ENZYMATIC HYDROLYSIS OF FREE FATTY ACIDS FROM SHEA BUTTER FOR USE IN COSMETIC INDUSTRY

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MEng/SEET/2017/ 7546

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF ENGINEERING IN THE DEPARTMENT OF CHEMICAL ENGINEERING

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ABSTRACT

Shea butter is a good form of vegetable oil extracted from the fruits of shea tree. This research is aimed to hydrolyse free fatty acids from shea butter oil for cosmetic production by extracting shea butter, producing Free fatty acids from shea butter oil through enzymatic hydrolysis and determining the optimum operating conditions. The Shea kernel was transformed into shea butter oil through mechanical screw expression method. Enzymatic hydrolysis of shea butter oil was carried out using lipase as enzyme, this was done by adding 30ml of phosphate buffer solution with a pH of 7.0 and 0.1g of lipase to shea butter oil of 5ml in a conical flask. The solution was stirred using magnetic stirrer and allowed to stay for 15minutes, three (3) distinctive layers were observed and subjected to further analysis. Shea butter was characterized and the results obtained indicated 0.915 Specific Gravity (S.G), 12.44% free fatty acid (FFA), 15.96 Acid Value (AV), 7.46MEq/100g of peroxide value (PV), 189.93 Saponification Value (SV), and 83.39 Iodine Value (IV) Shea butter oil was characterized and result of 50.2% oil yield, 2.64 MEq/100g of peroxide value (PV) and 2.72% of Free Fatty Acid (FFA) were obtained and compared with General Services Administration (GSA) standard of ≥50% oil yield; ≤ 5MEq/100g of PV and ≤ 3% free fatty acid. The optimization of enzymatic hydrolysis of Shea Butter was carried out using DOE 13.2.0 and optimum process parameters of enzyme concentration of 0.40g/mol, temperature of 34.04^{O}C and time of 107 min were obtained and the best optimum value of free fatty acid (FFA) at 56.38%. These process parameters were validated experimentally in the laboratory given the FFA value of 56% with the error of 0.65% which is close to allowable error of 0.5%. The FFA of 56% was further subjected to FT-IR and GC-MS, the result from FT-IR shows the functional groups of unsaturated and saturated fatty acids. These functional groups later depicted from the GC-MS to be 45.43% oleic acid (unsaturated) and 53.80% stearic acid (saturated) with traces of 3.36% α- Amyrin acid.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction

Shea butter is a form of vegetable oil that is extracted from the fruits of the Shea tree (*Vitellaria Paradoxa*). The Shea tree is a major component of the forestry parkland in the dry zone of sub Saharan Africa. It is also the major indigenous oil producing plant in this sub Saharan region (Lovett, 2005). Natural vegetable oil and fats have become very important sources of dietary energy, antioxidants, biofuels and raw materials for the manufacture of industrial products. They are used in food, pharmaceutical, cosmetics as well as oleo chemical industries (Okullo *et al*., 2010). Shea butter is traditionally used as a source of vegetable fat for cooking or as a moisturizer for dry skin during the Harmattan season (Loveth, 2005). One of the highest quality Shea butter in the world is found in abundance in Nigeria and it is sold at relatively low prices. Shea butter is popularly known as '*Kadanya'* in the northern part and '*Ori'* in the south-western part of Nigeria. It is soft and creamy and has a beige colour. Other traditional uses for Shea butter include soap making, medicine, walls water proofing and fuel for lamps.

The extraction of Shea butter in Africa has been an ongoing practice for years and over 2.5 million metric tons (MMT) is locally extracted annually in Africa (Lovett, 2005). In Nigeria, Shea trees grow in an extremely vast areas of Niger, Nasarawa, Kebbi, Kwara, Plateau, Benue, Kogi, Oyo, Ondo, Kastina, Kaduna, Adamawa, Taraba, Borno and Sokoto States (Ajala, 2016). The production of soap from Shea butter (triglycerides) and alkalis has been achieved for more than two thousand (2000) years by a process known as saponification. It is the alkaline hydrolysis of triacylglycerol (TAG). The hydrolysis reactions produce the fatty acids that form the basis for most oleo-chemical

processes (Tiamiyu *et al*., 2014). Since oils and fats make up the primary feedstock, a valuable by-product is glycerol. Glycerol and fatty acids are widely used as raw materials in food, cosmetics and pharmaceutical industries, hence their production from oils. Physical and chemical methods have extensively been employed as the basis for the Production of fatty acids (Tulashie *et al*., 2020).

Fatty acid is usually a mixture of oleic, stearic, palmitic, linoleic and arachidic acids with oleic and stearic acids predominating and together constituting about 85% of the fatty acid content of Shea butter (Saba, 2018). Over the years, several research studies have been conducted to develop various techniques for the production of fatty acids. Some of these methods include enzymatic hydrolysis, sub-critical and super-critical water, and KOH catalyzed hydrolysis of Esters also known as Saponification (Tiamiyu *et al*., 2014). It has been observed and proven over time that high temperatures and pressures usually accompany chemical catalyzed hydrolysis of oils and fat (Tulashie *et al*., 2020). Therefore, this research seeks to optimize the production of free fatty acid from Shea butter through Hydrolysis using Lipase as the most suitable enzyme because of its availability.

1.2 Aim and Objectives of the Study

The aim of this research is to hydrolyse free fatty acids from Shea butter for purpose of cosmetic production and this would be achieved through the following objectives:

i. Extract Shea butter from mechanical screw expression of Shea nut and determine the physicochemical properties of Shea butter.
ii. Produce Free-fatty acid from Shea butter through Enzymatic Hydrolysis and to determine the optimum operating conditions for the reaction.
iii. Conduct kinetic studies on the Enzymatic Hydrolysis of Shea butter.

iv. Determine the properties of Free-fatty acid through Gas-Chromatography and Mass Spectrometry (GC-MS) analysis and FT-IR analysis.

1.3 Scope of the Study

The scope of this study is limited only to the hydrolysis of free fatty acids from shea butter for cosmetic production.

1.4 Statement of the Research Problem

The demand of quality free fatty acids for the cosmetics, pharmaceuticals and beverage industries is on the increase. Extraction of Premium quality free fatty acids in Nigeria is quite poor and this has made the competitiveness in the global market to be below par. Reproducibility of free fatty acids has been a major concern over the years that must be understudied.

1.5 Justification of the Study

There is need to investigate and to optimize free fatty acid production from Shea butter processing parameters to ensure:

1. Higher and consistent quality of free fatty acid
2. Achieve high premium in international market
3. To aid reproducibility.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Shea Tree

According to the study shown by (Saba *et al*., 2018) Shea trees are native of West Africa, wildly grown, perennial tree crops, and was dispersed to other parts of east and central Africa by humans, monkeys and birds. The availability stretches from western Senegal to North Western Uganda (Lovett and Haq, 2000; Enaberue *et al.*, 2011 and Saba *et al*., 2017). The Shea tree is either called *Vitellaria paradoxa or Butyrospernum parkii* or simply *V. paradoxa* in West Africa or *"nilotica"* in East Africa. Examples of countries where these trees are found include Senegal, Mali, Ivory Coast, Burkina Faso, Togo, Ghana, Benin, Niger, Nigeria, Cameroon and further east in Uganda, Sudan and Ethiopia. The Shea tree is estimated to cover about 1,000,000 - 3,000,000km^2 between western Senegal and North Western Uganda (Sanou and Lamien, 2011; Olife *et al.,* 2013; Tiamiyu *et al.,* 2014 and Saba *et al*., 2018). The Shea tree grows to a height of between 10 to 20 m. The cylindrical trunk has a circumference of between 0.5 m to 2.5 m, usually relative to the height of the tree. A typical Shea tree is shown in Plate I.

Plate I: Shea Tree. (Saba *et al*., 2018)

2.2 The Shea Fruit

Fruit from Shea tree is egg shaped (shown in Plate II) and made up of green epicarp, a fleshy pulp (mesocarp) and a relatively hard shell (endocarp) which encloses the Shea kernel depicted in Plate III. The germination of Shea nut lasts about one month. In favourable environment, phyenological germination takes place within 7 to 10 day. Its growth is very slow and seedlings take 2 to 3 yrs to reach field planting. The juvenile stage of Shea tree lasts very long, 15 to 20 years, hence the difficulty in its domestication. Flowering starts at about the age of 20 years and production reaches maturity at the age of 40 to 50 years. The Shea tree can live for more than 200 years (Jia, 2019).

Plate II: Different Shapes and sizes of Shea Fruits (Saba *et al*., 2018)

Plate III: Shea Nuts (Saba *et al*., 2018)

Plate IV: Fresh and Dried Shea Kernels (Saba *et al*., 2018)

The flowers are a greenish-yellow colour and appear in groups of approximately 30 to 40 during the flowering season, which is between December and March (Maranz and Weisman, 2003). According to (Tiamiyu *et al*., 2014) and Gunstone (2002), average production of Shea tree is between 15 to 20 kg of fresh fruit per tree and about one tree in three is productive in each year. On average, 50 kg of fresh nuts give 20 kg of dry kernels (Plate IV), which contain 40% to 55% of fat.

2.2.1 Picking of shea fruits

Shea fruit picking is done mostly by children and rural women in the community where Shea trees are available (Lovett, 2004 and Perederic *et al.* (2020). The picking involves women and children leaving their homes as early as 5 am trekking distances up to 5 km for the Shea fruit picking Lovett. (2004). Large scale commercial and industrial picking is reported to be emerging as a result small and medium scale enterprises engagement in the processing of Shea products (Perederic *et al*., 2020).

The fruits picked are now collected inside basin and brought back home where they are kept in piles for 7 days before being processed. The de-pulping of the fruit involves either burying the fruit for the pulp to ferment and then fall off or crushed under foot or applying hand pressure to remove the pulp to obtain the Shea nut (Alonge and Olaniyan 2007).

2.2.2 Boiling of shea nut

Boiling of Shea nut as depicted in plate V is optional as it's still not practiced in some Shea producing communities in Nigeria. However, studies have shown the importance of controlled boiling of Shea nut prior to Shea nut drying and subsequently on the quality of Shea butter (Lovett, 2004; Maranz *et al.*, 2003 and Hossain *et al.*, 2012). The benefits of Shea nut boiling are stopping enzymatic browning, denaturation of proteins binding the fibre and holding the moisture and lipids thus making the fresh nuts easier and faster to dry. It also increases the availability of total fat content available for extraction. Lovett, (2004) also showed that in denaturing the lipase enzymes which may cause oxidation of kernel lipids during germination of the living seed, boiling can also reduce FFA but can also increase susceptibility to development of Peroxides due to reduction of Phenolic antioxidants compounds in the Shea kernel.

Plate V: Shea nut boiling before drying (Saba *et al*., 2018)

2.2.3. Drying of shea nut

Shea nut is dried by sun or with the aid of traditionally clay moulded oven and in some places solar panel dryers.

2.2.4 Sun drying

Sun drying is practiced among rural communities scattered all over the Shea belt (Lovett, 2004) and it may take three to four weeks before the nut is dried to constant weight. More recent development involve fabrication of wire meshed bed dryers (Saba *et al*., 2017), solar panel dryers (Aculey *et al.*, 2012).

Plate VI: Sun Dried kernels on Wire Meshed Bed (Saba *et al.*, 2018)

This method of drying even though preferred to traditional oven drying has a drop back of long drying time, formation of higher peroxide value and higher FFA (Saba *et al*., 2017 and Lovett, 2004).

2.2.5 The traditional oven

Traditional oven is moulded from clay mixed with grasses and a solution of *Parkia biglobosa* (Saba *et al*., 2017). It is about 2 m high and 0.75 m in diameter, mid-way into the height of the oven it is decked (Plate VII) and the decking is perforated (Plate VIII) to allow percolation of heat. The heating source is usually a blend of Shea shell, rice husk and firewood. The drying time takes about four day at a temperature of 45 ^{o}C to 60 ^{o}C (Saba *et al*., 2017). The fresh nuts are then fed into the oven and heat applied with occasional turning to avoid burning (Plate VIII).

Plate VII: Traditional Oven under Construction (Saba *et al.*, 2017)

Plate VIII: Internal Perforation of the Traditional Oven (Saba *et al.*, 2017)

The problems associated with this method of Shea nut drying are consumption of firewood, deposition of carbon on the Shea nut and adsorption of smoke which was thought to contain polycyclic aromatic hydrocarbon (PAH) and is reported to be carcinogenic (Lovett, 2004).

2.2.6 The shea kernel

Shea kernels are obtained after shelling the dried Shea nut either with stones, hammers or wooden pestle and mortar (Plate IX) (Alonge and Olaniyan 2007). The mixture of broken shells and the kernel is then winnowed as described by (Gunstone, 2002).

Plate IX: Women shelling Shea nut (Saba *et al*., 2017)

2.2.7 Pre-treatment of shea kernels

For the oil cells to be closer to the wall of Shea kernel, the kernel was roasted at 95 ^{o}C and kernel roasting has potentials of splitting up the Shea cells for more oil to be release during extraction. The major draw-back is also introduction of Polycyclic Aromatic Hydrocarbon (PAH) and high FFA when the RT is not controlled (Lovett, 2013). According to Saba *et al*. (2018), the high the roasting temperature the shorter roasting time taken for the required heat to reach the centre of the kernel.

2.3 The Shea Butter

Shea butter is a vegetable fat extracted from the kernels of the fruit of Shea tree (*V. paradoxa* or *nilotica*). It is a mixture of fatty acids usually oleic, stearic, palmitic, linoleic and arachidic acids, but oleic and stearic acids predominate and constitute about 85 -90 % of the fatty acid content of Shea butter (Olaniyan and Oje, 2007; Julius *et al.*, 2013; Warra and Ahmad, 2014).

2.3.1 Extraction of shea butter

The extraction of Shea butter is older than modern civilization and was initially done at the village levels and more recently also at industrial scale.

2.3.2 Methods of shea butter extraction

There are four major methods of Shea butter extraction namely; Traditional extraction process, Mechanical Pressing/ Extraction, Solvent extraction process and Biochemical (Enzymatic) extraction process.

2.3.2.1 Traditional extraction method

The traditional extraction method is either by direct boiling of the Shea paste or kneading the paste before boiling the curd (Babu *et al.*, 2011) reported that the traditional system of extraction is associated with low productivity of Shea butter extraction (Ferris *et al*., 2001) also reported that this method of Shea butter extraction is cumbersome, laborious and time consuming demanding a lot of physical energy from the processors. An investigation by (Hall *et al.*, 1996) also reveals that the production of 1 kg of Shea butter is estimated to take about 20 -30 hrs from collection of the nuts to Shea butter production at an efficiency of 25%. Studies by (Chibor *et al.*, 2017); (Saba *et al.*, 2017); (Obibuzor *et al*, 2014); (Chukwu and Adgidzi, 2008); obtained results of analysis of Shea butter extracted from this method to be poor and inconsistent in quality parameters (FFA = 13.23%, PV = 7.921%, iodine value = 80.21%, oil content, saponification value = 185.26%, refractive index, unsapofiable valu).

Furthermore, this method of extraction makes use of large quantities of wood fuel and water, which are collected by women and carried over long distances. This produces a significant drain on scarce resources in the semi-arid areas where Shea grows (Lovett, 2004). In another study (Hall *et al*, 1996) showed that between 8.5 kg to 10.5 kg of wood is consumed in the production of 1 kg of Shea butter. This method of traditional production differs from place to place. The different methods of traditional Shea butter production are pictorially described in Figures 2.1 to 2.4 respectively. As a result of

increase in demand of the Shea kernels and the Shea butter (Williams and Isemede 2015), efforts are made through research to increase the yield and quality of Shea butter by several unconventional methods (Didia and Iddrisu 2018); (Shehu *et al.,* 2017) and (Obeng *et al.,* 2010).

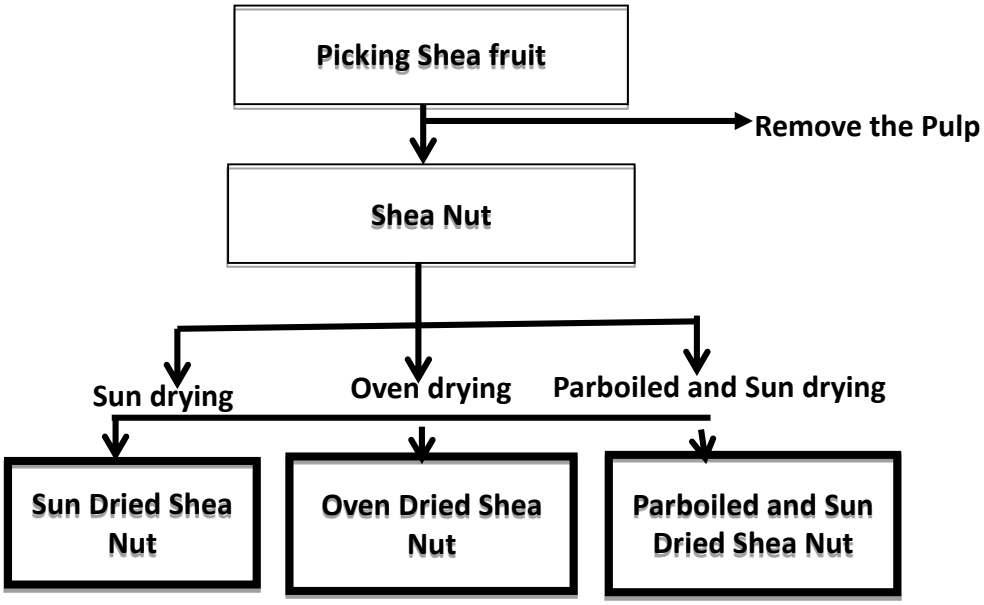

Figure 2.1: Steps showing procedure for obtaining Sheanuts from Shea fruit (Obibuzor *et al*., 2014)

Each of the Shea nut obtained from Figure 2.1 are processed according to the following steps shown in Figures 2.2 to 2.4.

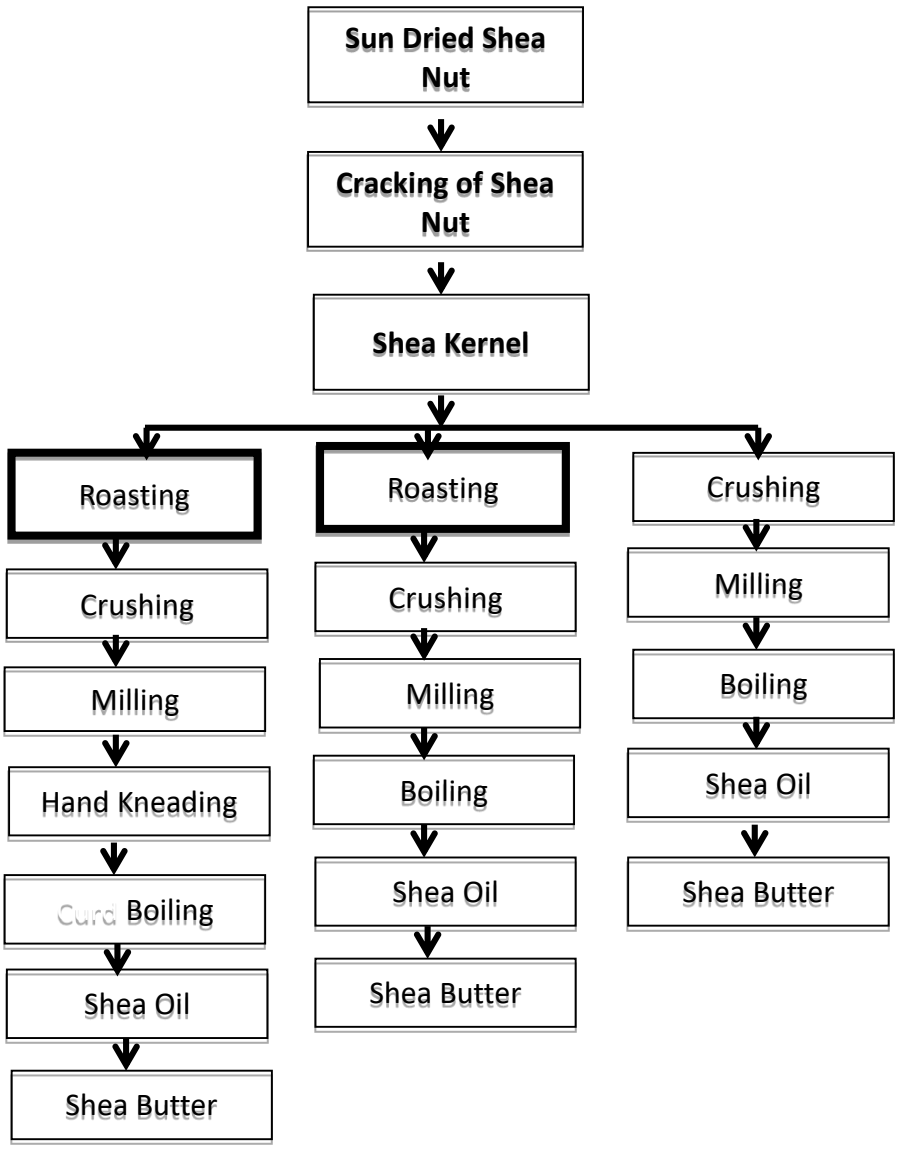

Figure 2.2: Existing Traditional Process Routes for Shea Butter Production from Sun Dried Shea Kernel (Chukwu and Adgidzi, 2008)

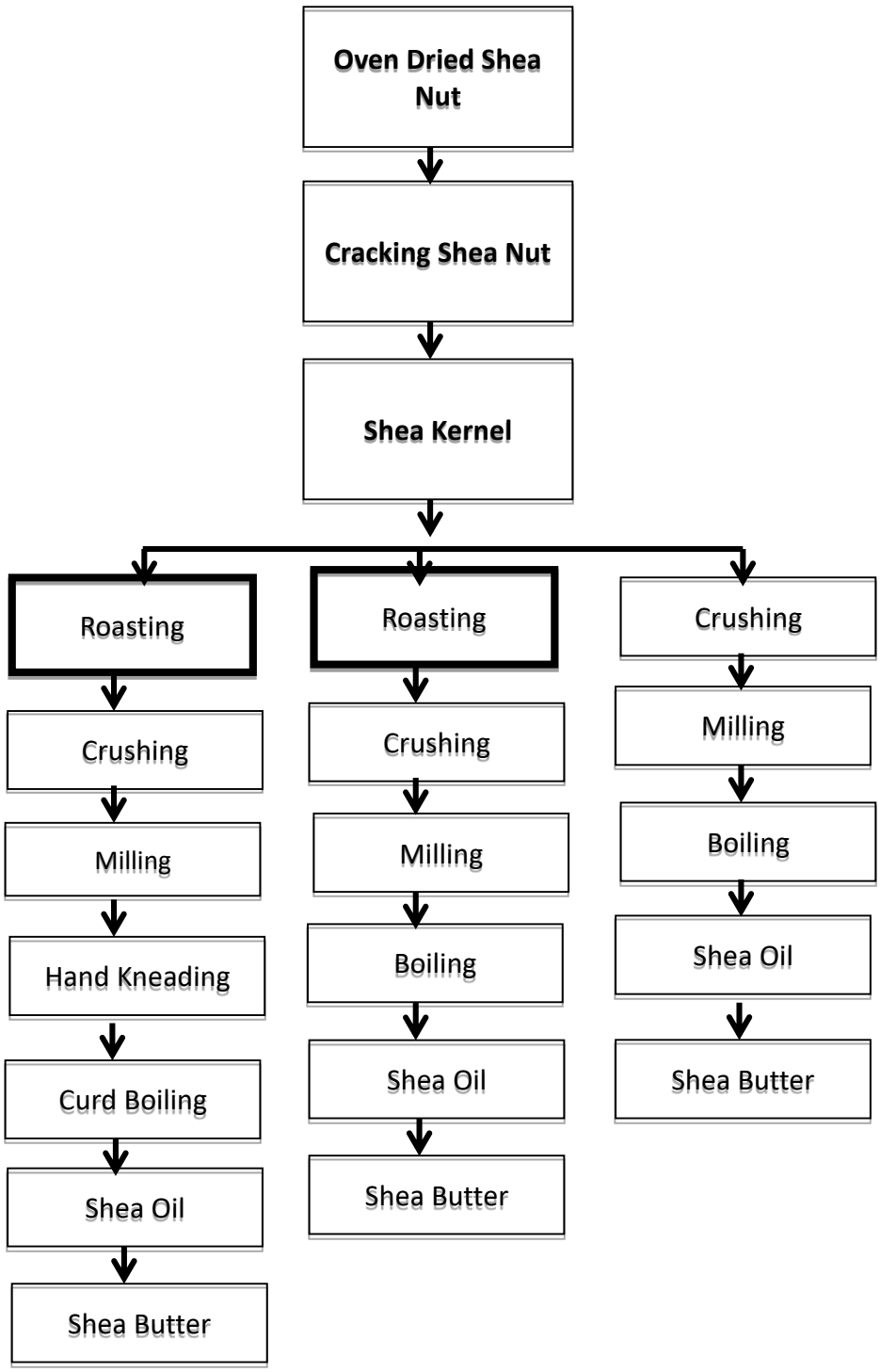

Figure 2.3: Existing Traditional Process Routes for Shea Butter Production from Parboiled and Sun Dried Kernel (Chukwu and Adgidzi 2008).

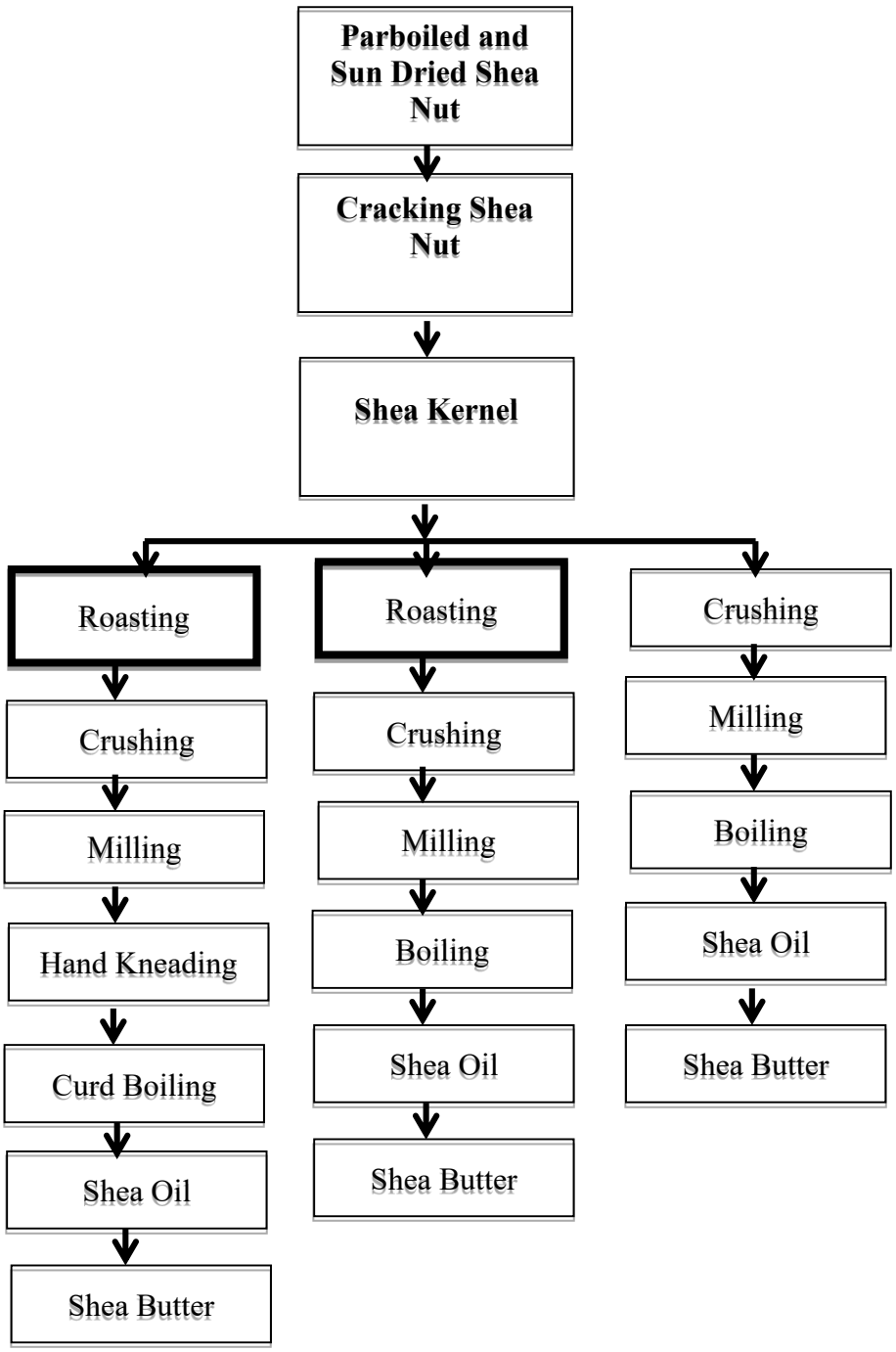

Figure 2.5: Existing Traditional Process Routes for Shea Butter Production from Traditional Oven Dried Shea Kernel **(**Chukwu and Adgidzi, 2008)

2.3.2.2 Mechanical pressing method

The purpose of the pressing process is to isolate the oily phase from the solid phase of the seed material through applied pressure. Two different pressing techniques are used for the separation of oil from the seed. In large-scale facilities, screw oil expeller

powered by an engine/electric motor, frequently after heating, the coarse ground Shea kernel is used to extract Shea butter. Screw-press is used in small-scale Shea butter extraction; here pressure is usually applied vertically on the milled Shea kernel, mostly by means of hydraulic press or manually turned screw (Lovett, 2004). In these techniques it is not possible to reduce the oil content to less than 5% oil in the press cake. Nevertheless, this method has the advantages of lower maintenance costs and investment, obtaining higher oil quality like virgin olive oil and it is easier to refine as it has less minor contaminants such as phospholipids (Lovett, 2004). In this process purification can be made by sedimentation. Moreover, consumers are fascinated in less processed food and thus only interested in oils extracted by a screw press and purified by filtration or sedimentation without the use of solvent or refining (Julius *et al.*, 2013).

2.3.2.3 Chemical (Solvent) extraction method

Solvent extraction is a process that makes use of organic solvents such as straight chain hydrocarbons, alcohols, chlorinated hydrocarbons and ketones to recover the oil from the sources. Kernel pre-treatment, oil extraction and solvent recovery are the most important parts of the extraction. The techniques for solvent extraction of nut are analogous to that of seeds (soybeans). Usually, the kernels are cracked into piece and conditioned to 10 – 11 % moisture at 70 ^{0}C and then flaked. At times, flaked nuts are cooked again before they are converged to the extractor. In the extractor, the lipid is separated by means of a solvent. The improvement of the solvent extraction process has enhanced oil recovery and has made it economically attractive to process seeds comparatively low in oil content; and this process is recommended if it is necessary to reduce the residual oil lower than 2 % (Julius *et al.*, 2013 and Matthäus, 2012).

In most instances, hexane is used as solvent, because it has good oil solubility at moderately low temperature, has a suitable boiling temperature, is cheap, noncorrosive to metals, does not react chemically with the oil, stable under the process conditions, immiscible with water, improves the separation of water from the seeds and it is easily and totally removed from the residue with low energy input and without impairment of the raw oil (Matthäus, 2012). Asuquo *et al.* (2010) reported that the solubility of Shea butter found to be higher in hexane than petroleum ether and Carbon tetrachloride. Furthermore, in a study by Alenyorege *et al*. (2015), hexane has maximum value of extraction constant with rapid efficient and maximum extraction of oil than acetone, benzene, and chloroform and petroleum ether in peanut, which has much similar physical properties with Shea kernel. Under most processing conditions, to ensure a comprehensive and quantitative recovery of tissue total lipids, a solvent system comprising of varying proportions of polar and non-polar components may be used. Due to the environmental problems, high costs involved and lack of technical skills in developing countries, solvent extraction method is not usually used in domestic Shea butter extraction. Alternatively, a large proportion of cosmetics Shea butter usually has been obtained through solvent extraction in a highly refined form (Alenyorege *et al.*, 2015).

2.4 Uses of Shea Butter

Shea butter is a natural plant extract with exceptional moisturizing and healing properties. Its usage therefore cuts across many human endeavours. It is used for medicinal purposes in the treatment of eczema, dermatitis, dry skin, dry scalp and hair, psoriasis, cracked skin, cracked bleeding skin, cuticle repair, Itchy skin, muscle fatigue, tension and aches, rough heels, rheumatism, arthritis, sunburn relief, skin rashes, diaper rash, scars, surgical incision and scars, skin blemishes, dark spots and stretch mark

minimization, wrinkle, fine line reduction, reactivation of collagen synthesis, Increase skin balance, elasticity and tone, eliminates dull and greyish complexion (Babu *et al.*, 2011). Stretch marks during pregnancy, wrinkle formation, dry, chapped, itchy skin and skin peeling. Other uses are in the protection of skin, confectionaries, chocolates, baking, cooking, cocoa butter substitute, greases, lubricants and Biodiesel, (American Shea Butter Institute, 2016).

2.4.1 Triglyceride fractions

The triglyceride component of Shea butter is made up of glycerol back bone and fatty acid as the side chain components. Since the fatty acid chain in Shea butter are many, different arrangement of Stearic-Oleic-Stearic (SOS), Stearic-Oleic-Oleic(SOO), Palmitic-Oleic-Stearic (POS), Palmitic-Oleic-Palmitic (POP) are possible, with their respective average composition of 40 %, 27 %, 6 % and 1 % of the fatty acid on the back bone i.e. glycerol in butter (Alander, 2004). The availability of these acids in varying proportions in Sheabutter is a function of the source of the Sheanuts (Di-vincenzo *et al.*, 2005; Njoku, 2006 and Zhang *et al.*, 2014).

In a study by Di-Vincenzo *et al.* (2005) it was found that Shea butter contains relatively high amounts of saturated fatty acids compared to other seed oils, and this accounts for its solid to semi-solid state at room temperature. Shea butter fatty acids were found to vary across the African countries (Nahm, 2011).

In yet another study, Lipp *et al*. (2001) observed that, unlike in Shea butter, the cocoa butter's tricylglycerides are composed of POP, PLP, POS, POO, PLS, SOS, SOO, SLS and Oleic-Oleic- Oleic (OOO) among which POP (18.3 %), POS (42.1 %), and SOS (26.4 %) are the major triglycerides. This composition compares favourably with those found in the studies of Alander (2004). Although the major fatty acids showed some

degree of variation, the profile of both cocoa and Shea nut butters were characterized by their high levels of stearic and oleic acids.

Table 2.1: Fatty Acid Compositions (%) in the Two Different Species of Shea Butter

Fatty Acid	Carbon and double bond present	East Africa(Uganda) species Nilotica	West Africa (Ghana) species Mangifolia/paradoxa
Palmitic Acid	(16:0)	4.4	3.9
Margaric acid	(17:0)	Trace	Trace
Stearic Acid	(18:0)	30.7	43.2
Oleic Acid	(18:1)	57.4	44.3
Linoleic Acid	(18:2)	5.8	6.3
Linolenic Acid	(18:3)	0.4	0.4
Arachidic Acid	(20:0)	0.8	1.2
Eicosenoic Acid	(20:1)	0.3	0.4
Docosanoic Acid	(22:0)	0.1	0.2
Tetracosanoic Acid	(24:0)	0.1	0.1
Total saturated Acids		*36.1*	*48.6*
Total mono-unsaturated Acids		57.7	44.7
Total poly-unsaturated Acids		6.2	6.7
Total unsaturated Acids		63.9	51.4

Source: (Hossain *et al.*, 2012).

2.4.2 Chemical composition of shea butter

The chemical composition of Shea butter is majorly influenced by two major factors;

i. Regional variability

ii. Post-harvest processing

Apart of Shea butter been majorly made up of a mixture of fatty acids, Esuoso *et al.* (2000) also stated that Shea butter is approximately 90% triglycerides by mass and the balance i.e. 10% unsaponifiable fraction. The triglyceride components of the Shea

butter are responsible for its emollient properties, while the unsaponifiable fractions are responsible for the healing properties and this constitutes the bioactive substances in Shea butter. These bioactive substances include hydrocarbons, tocopherols, sterols and alcohols (Esuoso *et al.*, 2000). The fatty acids are usually oleic (18:1), stearic (18:0), linoleic (18:2), palmitic (16:0) and arachidic acids, but oleic and stearic acids predominate and constitute about 85 -90%, linoleic about 5-10%, palmitic about 4% and arachidic less than 2% of the fatty acid content of Shea butter Alander, (2004); Olaniyan and Oje, 2007).

2.4.3 Unsaponifiable fraction

The unsaponifiable fractions of Shea butter even though small compared to the triglyceride component of Shea butter was found to be responsible for the healing properties of Shea butter (Nahm, 2011). They dissolve in fat and are insoluble in aqueous solution but soluble in organic solvent after saponification according to Hamilton and Rosell (1986). The amount of unsaponifiables in Sheabutter ranges from 4 to 11 % by weight, while typical vegetable oils and fats contain lower levels (0 – 2 %), Hamilton and Rosell, (1986). Nahm, (2011) and (Quainoo *et al.,* 2012) pointed out that these unsaponifiables of plant oils and fats have been subjects of intense research due to their various bioactivities including as antioxidant, antimicrobial and anti-inflammatory properties.

Alander, (2004), showed that the unsaponifiable fractions of Shea butter is dominated mostly by triterpene alcohols, followed by hydrocarbons, sterols, and other minor components such as vitamin E. According to (Itoh *et al.,* 1974), Sheabutter's unsaponifiable fraction was found to be composed of 75 % of triterpene alcohols which are usually esterified with cinnamic acid or fatty acids, 18 % of less polar compounds

such as hydrocarbons, 5 % of sterols and 2 % of methyl-sterols. But Lipp *et al.* (2001) in yet another study observed that the unsaponifiable fractions consist of 65 % of triterpene alcohols, 27 % of hydrocarbons and 8 % of sterols. (Alander 2004) also in a study reviewed that some threats contain hydrocarbons (karitenes) made up of 2 to 5 % of total unsaponifiables with lower amounts of tocopherols. Tocopherols in Shea butter were extensively studied by Maranz *et al.* (2003), and the work reveals that the total tocopherol content ranged from 0.0029 - 0.0805 % of the total Sheabutter. Four isomers of tocopherols (α, β, γ, and δ) were isolated among which α-tocopherol was found to be the major tocopherol (64 % of the total tocopherols).

However, Peers (1977) found out that the triterpene alcohols and sterols were mostly esterified to cinnamic and fatty acids. The composition of triterpene alcohols in Shea butter was studied by Itoh *et al*. (1974) and Peers (1977). Itoh *et al*. (1974) found α-amyrin as the major triterpenes (46 - 54.6 % of total triterpene alcohols) followed by butyrospermol (12.3 – 26 %), lupeol (16 – 17 %), β-amyrin (7.1 – 8 %) with the minor amounts of γ-taraxasterol, taraxasterol, parkeol, and cycloartenol. On the other hand, Peers (1977) found the major triterpene alcohols were α-amyrin (27.6 % of total triterpene alcohols), butyrospermol (26.0 %), lupeol (22.6 %), β-amyrin (10.6 %), and germanicol (9.4 %) with the minor levels of 24-methlyene-lanost-9(11)-en-3-ol, parkeol, dammaradienol, and 24-methylene-dammarenol. The composition of phytosterols in Shea butter was again studied by Itoh, Peers (1977) and Badifu (1989), and it was found that the major sterols were α- spinasterol (43 % of total sterols) Δ-7-stigmasterol (11 %), and 24-methyl-choest-7-enol (6 %). Peers (1977) on the other hand observed that α-spinasterol (50 % of total sterol) and Δ-7- stigmasterol (50 %) were the major sterols while Badifu (1989) found the β-sitosterol (68 % of total sterol), stigmasterol (20 %), and campesterol (11 %) were the major phytosterols.

Shea butter also contains other minor bio actives and phenols within the unsaponifiable fraction. Maranz *et al.* (2003) showed that the value of phenols obtained from Shea butter is about 3 % of the 100 % present (4000 ppm) in the kernel. This implies that the bulk of the phenol is lost during hexane extraction. These results suggest that the phenol fractions are highly polar and thus far less soluble in fat.

2.5 Regional Variability of Shea Butter's Characteristics

The variability of Shea butter composition (fatty acid) is region influenced because of differences in bioclimatic conditions of temperature and rainfall (Quainoo *et al.,* 2012 and Abdulahi *et al*., 2015). The West African species *Vitellaria paradoxa* is one in which the Oleic and Stearic acid contents (% composition) are similar and this explains why it is in semi-solid to solid state at room temperature and most preferred in the chocolate industries in Europe. The East African species has higher Oleic acid than Stearic acid and as such is in liquid state at room temperature and mostly used by cosmetic industries. The latter is currently not available in commercial quantity because of political instability in the regions where it is produced (Sudan, Uganda), even though it attracts more premium (Hossain *et al.,* 2012; Ferris *et al.,* 2001). Table 2.2 shows fatty acid composition of some selected African Shea butter producing countries.

Table 2.2: Regional variability of fatty acid composition (% Total FFA) of sheabutter

Origin	Palmitic	Stearic	Oleic	Linoleic
Carbon: double bond	16:0	18:0	18:1	18:2
Benin	3.8	44.1	43.8	6.65
Burkina Faso	12.1	42.5	39.3	4.5
Burkina Faso	3.3	43.5	44.5	5.9
Cote d'Ivoire	6.6	46.8	51.4	8.4
Mali	3.3	43.3	44.6	6.0
Mali	19	31.1	42.6	5.7
Nigeria (Northern)	4	46	41	7
Nigeria (Western)	3.4	43.8	44.3	5.8
Nigeria (Northern)	3.2	38.9	47.5	6.5
Chad	4.2	22.5	68.0	4.9
Uganda	4.2	28.9	57.8	6.3
Uganda	6.5	26.4	59.3	6.2

Source: (Nahm, 2011)

2.5.1 Regional variability of triglyceride composition

Regional variation of triglyceride composition was also investigated by Di-Vincenzo *et al.* (2005) using Ugandan samples and the results showed that major triglycerides are SOS (19.87 %) SOO (33.39 %), OOO (19.07 %), while the same triacylglycerides in samples from Mali, Burkina Faso, and Nigeria were 40 %, 26 %, and 10 % respectively.

2.5.2 Regional variability of unsaponifiable fraction

The bioclimatic conditions (rainfall and temperature) of regions also influence the unsaponifiable fractions of Sheabutter. Maranz *et al.* (2003) observed that as the environmental stress on the Shea tree increases, the concentration of the phenol in the Shea kernel increases. This was seen when Shea kernels from hot and dry areas of Lake Chad were compared with kernels from wet and colder areas of Guinea and West Cameroon. The study also revealed that because of solubility of Gallic acid in hexane, only a small amount of phenols can be obtained from the Shea butter extracted using hexane compared to the kernel. This makes the regional variation of unsaponifiables (phenols) in Shea butter very low as compared with the kernels (Nahm, 2011).

Tocopherol content, especially α-tocopherol, which is a dominant tocopherol of Shea butter, was directly correlated with temperature. The concentration of α-tocopherol in Shea butter from hot, dry areas (N'Djamena in Chad) was higher (414 μg/g) than the butters that originated from cooler areas of Northern Uganda (29 μg/g) (Maranz and Wiesman, 2003).

In yet another study conducted in Mali, Burkina Faso, Nigeria, and Uganda by Di-Vincenzo *et al.* (2005) also confirmed variation of triterpene alcohols in the unsaponifiable fraction. The study found that the West African Shea butters from Nigeria (12.6 %), Mali (9.6 %) and Burkina Faso (7.1 %) contained more triterpene alcohols than the East African butters, with the Ugandan butters containing only 3.7 %.

2.6 Effect of Environmental Factors on Shea Butter Characteristics

The influence of environmental factors in Shea butter starts immediately the Shea fruits are matured and falls on the ground (Nahm, 2011).

2.6.1 Effect of extraction method on shea butter

The extraction of Shea butter involves series of steps and varies from region to region or community to community. But on a general basis, it involves pre-treatment of the Shea nuts and processing of the kernels to produce the Shea butter. The pre- treatment includes, picking of fruits when fallen to the ground, removal of the pulp, drying of the nuts in sun or oven, decortications of nuts, cleaning and sorting of nuts, roasting and cracking of the nuts. On the other hand, the processing of the kernels to Shea butter involves, crushing /grinding of the Shea kernels, kneading and rolling to produce the curd. The curd is then boiled with water or solvent and allowed to cool, the oil is skimmed and allowed to solidify and then packaged according to Julius *et al.* (2013). The differences in pre-treatment of Shea nuts and the extraction process are mostly

responsible for the differences and inconsistencies in the quality of Sheabutter Aculey *et al.* (2012).

Studies conducted by Mbaiguinam *et al.* (2007) also found that hexane extraction or the extraction method including the parboiling of the seeds yielded Sheabutter with almost half level of FFAs compared to Shea butter extracted without seed-boiling procedure. While the butters from hexane extraction method and the procedure involving parboiling of seeds showed acidity of 5.1 - 5.5, the butters extracted from sun dried nuts, with no boiling, showed much higher levels of acidity (10.3 - 10.6).

Also the choice of solvent has direct influence on the quality of the Shea butter as solvents selectively dissolve some of the bioactive (phenols, Vitamin E) in the Shea butter when separating the oil from the kernel. Temperature has tendencies of increasing the FFA (FFA) and PVs in Shea butter and subsequently these lead to the Shea butter with high levels of thermal destruction of oil cells and increase in lipase activity, rancidity values, as well as decreased palatability due to decreased ester value, (Lovett, 2004 and Nahm, 2011).

Effects of temperature, moisture content, particle size and Rt were also shown to influence the efficiency of Shea butter extraction according to Olaniyan and Oje (2007) and Obeng *et al.* (2010).

2.7 Acid Value (AV)

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil.

2.8 Enzyme

Enzyme is a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process; it catalyze all aspects of cell metabolism. According to Zhang, (2014), enzymes are proteins that act as biological catalysts. Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products.

2.8.1 Examples of specific enzymes

According to https://www.medicalnewstoday.comacc06/24/2021, *t*here are thousands of enzymes in the human body; here are just a few examples:

i. Lipases – a group of enzymes that help digest fats in the gut.
ii. Amylase – helps change starches into sugars.
iii. Maltase – also found in saliva; breaks the sugar maltose into glucose.

2.8.2 Enzymatic hydrolysis of Sheabutter

Many enzymes in the human body have an optimum temperature of approximately 40 ˚C. Normally, the lipase enzyme will hydrolyze fats most efficiently at a temperature of about 37 – 40 ˚C, as this is close to our human body temperature (37 ˚C) and matches the temperature of the digestive organs in which lipase acts, (https://www.ucl.ac.uk›lipaseacc6/24/2021).

2.8.3 Effect of time on lipase-enzyme at room temperature (30 ^{o}C) and agitation of 100 rpm

Enzyme is an organic substance that assists in lowering the time taken to breakdown complex hydrocarbon into component molecules; e.g. fats and oil into glycerol and free fatty acid, (https://www.britannica.com › science › enzyme).

2.8.4 The effect of temperature on lipase for the production of FFA at constant time of 60 min and agitation of 100rpm

What happens to lipids when heated? The investigating effect of temperature on the activity of lipase shows increasing temperatures from 0 ºC to around 45 ºC will reduce the time taken for the lipase to break down the fat. Over this temperature, the time taken will increase, perhaps the lipase will not work at all, (https://www.nuffieldfoundation.org6/26/2021).

Higher temperatures tend to speed up the effect of enzyme activity, while lower temperatures decrease the rate of an enzyme reaction. At higher temperatures, more molecules collide, increasing the chance that an enzyme will collide with its substrate. If the enzyme's shape changes, it cannot bind to the substrate, (https://education.seattlepi.com › science-experiments-dem).

2.8.5 Kinetics of enzymatic hydrolysis

Several mechanisn have been proposed for lipase catalysis reactions (Ramachandran *et al.*, 2002). The vast majorities of these mechanisms were developed for the case of soluble lipases acting on insoluble substrates (i.e. oil droplets dispersed in water). However, in the absence of diffusional limitations the validity of the aforementioned mechanism easily extends to include the most complex case of having the lipases present in immobilized states. The simplest kinetic model applied to describe lipase catalyzed reaction is based on the classic Michaelis-Menten mechanism as applied to emulsified oil water systems, (Ramachandra *et al.*, 2002). The kinetic steps can be represented schematically as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_{cat}} E + P + Q \qquad (2.1)$$

where E denotes the Enzyme, S the substrate (glycerides), ES the enzyme substrate complex and P and Q the products (hydrolyzed glycerides and free fatty acids). The rate

of formation of free fatty acids per unit volume of reacting fluid (r) can be represented in terms of this mechanism as:

$$r_v = V_{max}(\frac{S}{K_m}) + S \quad (2.2)$$

$$V_{max} = k_{cat}(E)_{tot} \quad (2.3)$$

$$K_m = k_{cat} + \frac{k_{-1}}{k_1} \quad (2.4)$$

where V_{max} is the rate observed when the lipase is saturated with substrate, k_m is the Michaelis-Menten constant, the bracket denote molar concentrations of the various species, and the subscript tot denotes the total amount.

In the case of feed stocks from natural origin which contain more one chemical species susceptible to lipase action (e.g. Butter fat), the Michaelis-Menten mechanism denoted as equation (2.1) may be extended in order to include competitive inhibition by every substrate, S with respect to each other. For extent of hydrolysis below 70%, a pseudo-zero order rate expression arises which takes the form.

$$r_{vi} = \frac{V_{max}(S_t)}{\sum_{j=1}^{N}(S)} \quad (2.5)$$

$$V_{max} = k_{cat}(E)_{tot} \quad (2.6)$$

The above equation is based on the following assumptions:

(1) the Michaelis-Menten constants, k_{mi} associated with every substrate S_i (out of N possible substrates) are approximately equal and

(2) all $(S)/K_m$ are very large compared to unity.

Three rate expressions were utilized based on Michaelis-Menten kinetics and a ping-pong bi-bi mechanism to fit the unresponsive experimental data for the total rate of release of fatty acids.

Model A $r_{vhyd,A} = \theta_{A,1}[G]$ (2.7)

Model B $r_{vhyd,B} = \frac{\theta_{B,1}[G]}{1+\theta_{B,2[G]}}$ (2.8)

Model C $r_{vhyd,C} = \frac{\theta_{C,1}[G]}{1+\theta_{C,2}[G]+\theta_{C,3}(G)^2}$ (2.9)

where, $r_{v,hyd,A}$, $r_{v,hyd,B}$ and $r_{v,hyd,C}$ correspond to the effective rate of reaction per unit volume (M/h) for the various models. (G), is the concentration (M) of glycerides moieties (accessible ester bonds and θ, represent lumped parameter related to the rate parameter appearing in the various Michaelis-Menten models (V, parameter, and Michaelis-Menten constant), these rate expressions are identical in mathematical form to those employed by (Ramachandra *et al.*, 2002). If the feedstock does not contain, initially significant amount of free fatty acids, then (G) can be determined by the following equation.

$\{G] = [G_{i,}\} + [FA]$ (2.10)

where $[G_{i,}]$ represent the initial concentration of glyceride bonds accessible hydrolysis and [FA] is the concentration of free fatty acids resulting from hydrolysis.

2.8.6 Deactivation kinetics

One of disadvantage and probably the most serious disadvantage of biocatalyst is that they lose their catalytic activities during the reaction. This phenomenon is well recognized in biotechnology and is called decay, inactivation, or denaturation of biocatalyst. Even if the life of a biocatalyst can be prolonged by its immobilization, it still loses its activity sooner or later. The catalytic durability of the biocatalyst during continuous operation is called operation stability. The operation stability of a biocatalyst is estimated by its half-life, which is the elapsed time at which the catalytic activity is reduced to half. The half-life is a very important parameter that governs the economic feasibility of the bioprocess concerned (Afaf, 2003).

Two methods are known to evaluate the deactivating catalyst during continuous operation. For the most common case continuous operation is performed so as to keep the conversion fixed (constant conversion). This requires decreasing flow rate as the enzyme activity drops gradually. In other method the flow rates (or the space velocity is kept constant throughout the continuous operation (constant feed rate or constant space velocity) to observe the decrease in the outlet conversion. The former method requires a precise feedback control strategy. Otherwise, trial and error in changing the flow rate is inevitable as the exact profile of the activity decay is unknown prior to the experiment. Because of the ease of experimentation, the later policy has been often applied in the biotechnological area (Ajala *et al*., 2016). Generally, deactivation rates are determined in the absence of substrate, but enzyme deactivation rates can be considerably modified by the presence of substrate and other materials.

Biocatalyst thermal stability is a fundamental aspect in the reactor performance. Despite this, most information on biocatalyst stability being gathered under nonreactive conditions, is of limited use, leaving aside modulation of enzyme inactivation by reagents and products has been studied and made explicit in reactor modeling ARSO, (2017).

Different mechanisms have been proposed to describe enzyme thermal inactivation. The simplest and most used is one stage first-order kinetics, which proposes the transition of a fully active native enzyme to a fully inactivated species in a single step. Such mechanism leads to a model of exponential decay.

$$\frac{\mathfrak{e}}{\mathfrak{e}_o} = \exp(-k_1 t) \tag{2.11}$$

Thermal inactivation is certainly more complex and series and parallel mechanisms have been proposed to describe it, (Ramachandra *et al.,* 2002). Models derived from

such mechanisms contain a high number of parameters, which are difficult to determine experimentally. However, a two-phase series mechanism usually represents well the phenomenon in terms of a limited number of parameters susceptible to reliable experimental determination. A model based on such mechanism is represented by equation (2.11).

$$\frac{e}{e_o} = [1 + A\frac{k_1}{k_1 - k_2}]\exp(-kt) - A\frac{k_1}{k_1 - k_2}\exp(-kt) \quad (2.12)$$

where r stands for enzyme activity, e_o is its initial value, t stands for time in seconds k1 and k2 are the transition rate constants in each inactivation stage and A is the specific activity ratio between the intermediate and initial enzyme stage. These models have been traditionally used to evaluate enzyme stability under non-reactive conditions (Ramachandra *et al.*, 2002), so that parameters obtained do not reflect the behavior in the presence of substrate and products as it occurs in the reactor. It has been postulated that any substrate that interacts with the enzyme during catalyst is a potential modulator of enzyme stability (Ramachandra *et al.*, 2002).

Therefore;

$$\frac{-d(E,J)}{dt} = k1,[E,J] \quad (2.13)$$

Kij = ki(1-nij) where nij represents the modulation factor of modulator J in inactivation stage i. for instance, in the case of an enzyme subjected to product competition inhibition, three enzyme species will exist: the free enzyme (E) and the secondary enzymes-substrate (ES) and enzyme-product (EP) complexes, among which the enzyme will be distributed during the course of catalysis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials, Chemicals and Equipment

The materials, chemicals and equipment used in this research work are shown in Tables 3.1, 3.2 and 3.3 respectively.

Table 3.1: Materials used for the Research

S/N	Materials	Quantity	Sources
1.	Fresh Shea nut	25kg	Gutsungi Village in Agaie, Niger state

Table 3.2: Chemicals used for the Research

S/N	Chemical	Quantity	Source
1.	AnalyticalGrade Sodium Hydroxide.	1000g	BDH chemicals England
2.	Analytical Grade Iso octane	2.5 L	Merck Chemicals Germany
3.	Analytical grade Phosphate Buffer Solution	2.5 L	BDH Chemicals England
4.	Analytical grade Glacial Acetic acid	1 L	BDH Chemicals England
5.	Analytical grade Chloroform	1 L	BDH Chemicals England
7.	Analytical grade Potassium Hydroxide	500g	BDH Chemicals England
8.	Analytical grade Sodium Thiosulphate Pentahydrate	25g	Guangdong Chemicals China
9.	Phenolphthalein	25g	Merck Chemicals Germany
10.	Iodine crystals	100g	BDH Chemicals England
11	Analytical grade Potassium Iodide	1 L	BDH Chemicals England
12.	Analytical grade Carbon Tetrachloride	1 L	BDH Chemicals England
13.	Water	Assorted	WAFT Analytical Lab, FUT Minna borehole Water
14	0.02N KOH in ethyl alcohol	500g	BDH chemicals England
15	Analytical grade n-Hexane	1 L	BDH chemicals England
16	Analytical grade Thymolphthalein in ethyl alcohol	25g	Merck Chemicals Germany

Table 3.3: Equipment used for the Research

S/N	Equipment	Model/year	Manufacturer
1	1 Wire meshed bed		Fabricated
2	2 Diamond Weighing balance	Sensitivity 500g	Taiwan
3	3 Digital Balance	A and P Co. LTd.	Korea
4	4 Mettler Toledo Balance	AL 204	China
5	5 Gallenkamp Hot plate	7B/1700A	England
6	Bomb Calorimeter	90/RN 20	Taiwan
7	Vari Mixer	No. 323	Denmark
8	Rosedowds screw press	S/N 905/1/1	UK
9	Automatic Sieve Shaker	D 403 Italy	Italy
10	Wooden pestle and mortar		Bida market
11	Solitaire Laboratory blender	VTCL India	India
12	Laboratory Oven	Euronic Model No: ES 9011	China
13	GC-MS Machine	7890A/5675C	USA
14	FTIR Machine		
15	Micro burette (2 ml with 0.01 ml intervals)		
16	Conical flasks (100 ml)		
17	5 ml pipettes		
18	5 ml Syringe		

3.2 Methodology

In this section, the methods used in the preparation of reagents for this research, collection of the Shea fruit samples, removal of the pulp is presented. The procedures for Shea nut processing into Shea kernel and the production of Shea Butter using mechanical screw expression are also presented. The preliminary experiment to establish the boundary conditions for the design of experiment using enzymatic hydrolysis to produce free fatty acid as its affect enzyme concentration, temperatures, time and constant Agitation speed are also shown in this chapter. The Steps followed to produce FFA samples from the optimum conditions of enzymatic hydrolysis were equally shown. Characterization of the Shea kernel, Shea butter and free fatty acid

samples produced based on quality parameters carried out are also presented and compared with those of literature in chapter two.

The research involves optimization of factors that has influence on the quality of free fatty acid using enzymatic hydrolysis method. These factors that influence free fatty acid optimization are: enzyme concentration, reaction Temperature, reaction time and constant agitation speed. This involves collection of matured and freshly dropped Shea fruits from the farms at Gutsungi and taking them to WAFT, FUT Minna where the removal of the pulp was made. The Shea nut was not boiled and was dried at certain temperature until the Shea nut has constant weight. The time frame expended in picking the fruits, pulp removal and washing was also noted. The production of Shea butter from Shea kernel using mechanical screw method was adopted while the responses monitored are % yield, Acid value, FFA, PV, Refractive index, Saponification value and unsaponifiable fraction. The optimum conditions of free fatty acid production were also generated from the design expert and also validated experimentally. The free fatty acid produced optimally by enzymatic hydrolysis was characterized using FTIR and GC-MS. The procedure of the methodology is summarized in Figure 3.1.

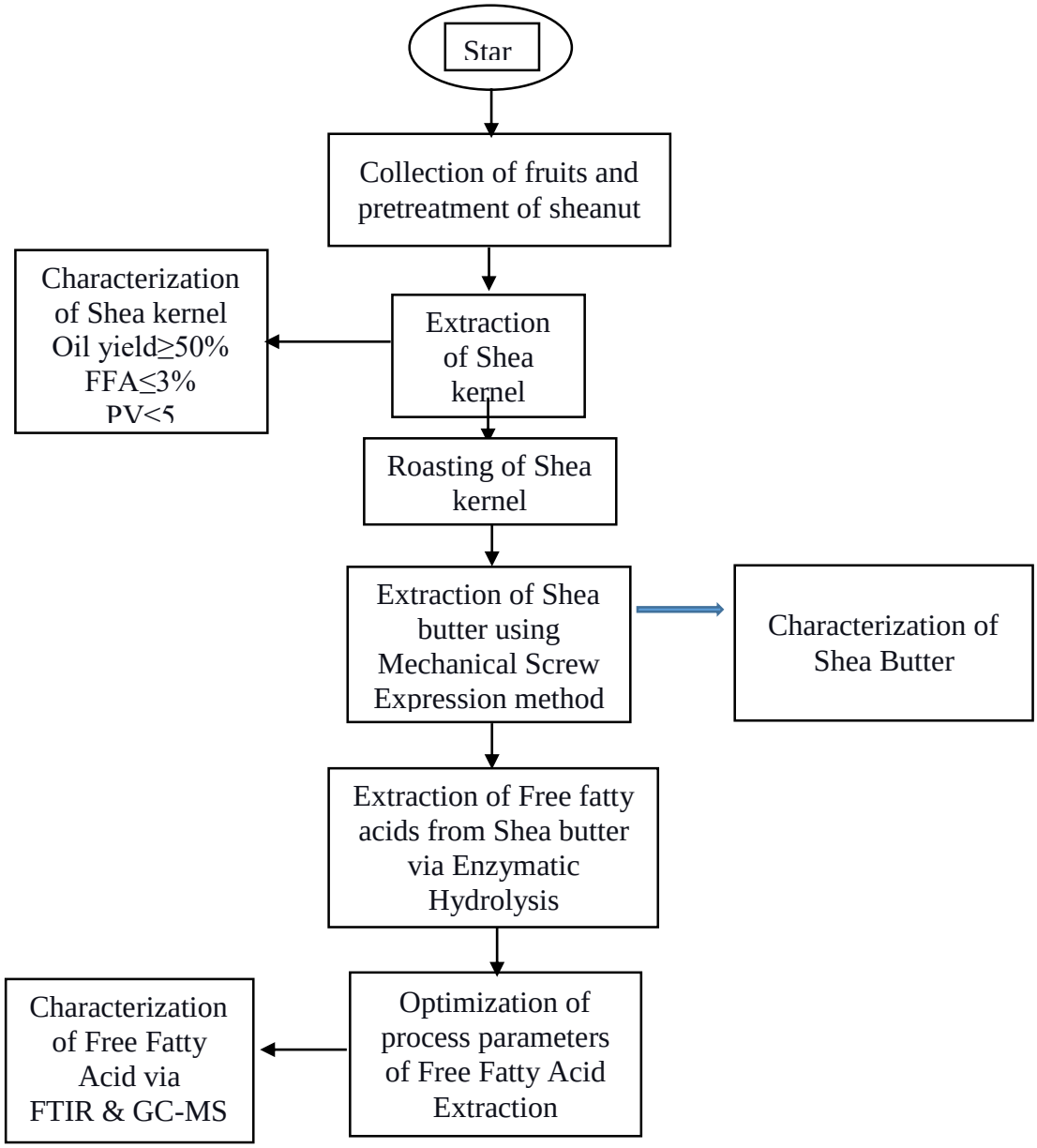

Figure 3.1: Flow diagram of the research methodology

3.2.1 Reagents Preparation

The reagents used in this research were all prepared from analytical grade chemicals as shown in Table 3.1 and were prepared as described in Subsection 3.2.1.3 to 3.2.1.14.

3.2.1.1 Potable water

The water used in the course of this research was obtained from the Water Resources, Aquaculture and Fishery Technology, Federal University of Technology, Minna, Niger

State, Nigeria. This water conforms to NIS 554: 2007 and was used for cleaning and washing of Shea paste. This potable water was also fed to distillers to obtain distil water used for reagents preparation, washing of glass wares before been used.

3.2.1.2 Preparation of distilled water

Distilled water used for the preparation of all the reagents were distilled from Manesty distiller in the agriculture laboratory of Water Resources and Aquaculture Department, Federal University of Technology, Minna, Nigeria. The distilled water was stored in corked plastic bottles as described in sub section 3.3.6.

3.2.1.3 Preparation of neutralized alcoholic diethyl ether

Neutralized alcoholic diethyl ether was prepared by mixing 25 ml each of diethyl ether with 25 ml of absolute ethanol in a 100 ml beaker. 1 ml of 1% phenolphthalein indicator was added to the mixture and neutralized with 0.1 N NaOH prepared as described in Subsection 3.2.1.5. The neutralized alcoholic diethyl ether tested neutral to litmus paper and was also used in sub section 3.3.6.

3.2.1.4 Preparation of 0.02N potassium hydroxide (KOH)

0.02N KOH in ethyl alcohol Weigh 5.6 g of potassium hydroxide, dissolve in distilled water and make up to 100 ml with distilled water (1N solution). It was Diluted 50 times with ethyl alcohol when required. n-Hexane and 1% phenolphthalein or thymolphthalein in ethyl alcohol Dissolve 1 g of either indicator was dissolved in 100 ml of ethyl alcohol and was used as described in sub section 3.3.4.

3.2.1.5 Preparation of acetic –chloroform solution

300 ml of glacial acetic acid was mixed with 200 ml of chloroform volume by volume in a 1 L flat bottomed flask. The content was thoroughly mixed manually to ensure

homogeneity. It was then stored in a 1 L measuring cylinder and used in the course of the analysis and was used in sub section 3.3.6

3.2.1.6 Preparation of 10% potassium Iodide

100 g of potassium iodide was weighed using a digital balance and was dissolved in 1000 mL of distilled water. The solution was stored in a glass bottle and used in sub section 3.3.3.

3.2.1.7 Preparation of 0.01N sodium thiosulphate

2.48 g sodium thiosulphate pentahydrate was weighed using a Metler toledo balance and poured into a 1000 cm^3 volumetric flask. 10 ml of distilled water was added to dissolve it. 0.1 g of Na_2CO_3 was added to increase its stability. Thereafter more distilled water was added to make it up to 1000 cm^3 mark. It was then filtered using 11 cm Whatman filter paper and then stored overnight in a dark glass bottle and used in sub section 3.3.3

3.2.1.8 Preparation of 0.5m alcoholic potassium hydroxide

28.05 g of potassium hydroxide was weighed using a Metler toledo balance into a 250 mL beaker and dissolved with 50 mL of distilled water. The solution was then transferred using a funnel into a 1 L volumetric flask and made up to 1000 cm^3 mark with absolute ethanol. The solution was then stored in a corked glass conical flask and used in sub section 3.3.5 and subsection 3.3.6.

3.2.1.9 Preparation of 0.025N sodium hydroxide (NaOH)

1.0g of Sodium Hydroxide pellets was carefully measured using metler Toledo weighing balance. It was then dissolved in 200 mL of distilled water in a 1 L volumetric

flask and then made up to 1 L mark and then store in a glass corked conical flask for use in sub section 3.3.2.

3.2.2 Collection of shea fruit

2.5 hectares of Shea tree farms was acquired at Gutsungi village as shown in plate XI in Agaie Local Government Area of Niger State, Nigeria, housing 40 Shea trees was used as an experimental farm to collect Shea fruits. The matured Shea fruits that had fallen was collected and kept in the open space/floor for 4 days. These collection was done daily between the hours of 7am to 10am daily to ensure consistency in timing of the Shea fruits sample collection throughout the period of collection (July to August). On each sampling day the Shea pulp was removed manually by applying hand pressure to expose the Shea nut.

Plate XI: Experimental farm where Shea fruits are collected Tree (Saba *et al*., 2018)

3.2.3 Preliminary Production of Shea Butter

Freshly de-pulped Shea nut were collected from Gutsungi village in Agaie local government area of Niger State, Nigeria. The nuts obtained from these locations were mixed, sorted and sprouted ones were discarded. From the remaining nuts, 80 kg was weighed using Diamond weighing balance (Plate XII) and sun dried for 14 days. The shell of the dried nuts was cracked using wooden pestle and mortar to expose the fresh kernel. The fresh kernels were again exposed to sunlight for further reduction of moisture content to between 5-6 %. 25 kg of the dried kernels was subjected to Mechanical screw machine shown in plate XIII. The oil collected was kept for 6h to allow the solid particles to settle and the clean oil was collected and allowed to congeal, packaged, refrigerated and then analyzed for physico-chemical properties.

Plate XII: Diamond Weighing Balance
Source: WAFT Laboratory, FUTMINNNA

Plate XIII: Rosedowns Mini 40 Screw Press
Source: National Cereals Research Institute, Badeggi (NCRI)

3.3 Characterization of Shea Nut and Shea Butter

The Shea butter produced in this research was characterized according to internationally recognized standards, AOCS: 1994 for FFA, ISO 3960: 2005 for PV, ISO 3657: 2005 for Saponification value, AOAC: 2000 for Unsaponifiable fraction, AOAC: 2000 for refractive index. The functional groups present were determined by Fourier infra-red transform spectroscopy (FTIR) and the constituent chemical composition was determined by Gas chromatogram-mass spectroscopy (GCMS).

3.3.1 Percentage oil yield determination and extraction efficiency

The percentage oil yield was determined by using the relationship below:

$$Oil\,Yield, Y = \frac{W_u - W_e}{W_u} \times 100 \qquad (3.1)$$

where,

W_u = Weight of unexpressed sample (g)

W_e = Weight of expressed sample (g)

The Extraction Efficiency, E_E is defined as follows

$$E_E = \frac{S_y}{T_{sy}} \times 100 \quad (3.2)$$

where,

S_y = Sample oil yield in percentage (%)

T_{Sy} = The total seed oil content in percentage (%) (Badifu, 1989)

3.3.2 FFA determination of shea butter

The FFA content of the Shea butter was determined using AOCS (1994) method. About 7g of the Shea butter was measured and poured into a 250ml flask in addition to 50ml neutralized alcohol. The mixture was titrated with 0.25N NaOH with vigorous shaking until permanent faint pink colour appears and was maintained for a period of 60 s. The millilitre of 0.25N NaOH used in the titration corresponds to the percentage FFAs present in the oil which was calculated by using the equation given below;

$$FFA(\%) = \frac{(V - B) \times N_f \times 28.2}{W} \quad (3.3)$$

where,

V = Volume of NaOH ethanolic solution used for titration (ml).

B = Volume of NaOH consume during FFA determination blank titration (ml)

N_f = Normality of NaOH factor

W = Weight of Oil sample (g)

Prior to this, a blank titration was carried out and 0.25N NaOH was used

3.3.3 Determination of peroxide value of shea butter

Two (2) gram of Shea butter sample was measured and transferred into a clean dried tube, 1 g of potassium iodine (KI) powder were added to the oil samples and 20 cm^3 of the solvent mixture (glacial acetic acid and chloroform in the ratio 2:1). Then the boiling tube was placed in different boiling water bath to boil the liquid mixture for

30seconds; the contents after boiling were quickly transferred into a flask containing 20 cm^3 of 5% potassium iodine (KI) solution. The tube was washed out twice with 25 cm^3 of distilled water. The resulting mixtures were then titrated with 0.002M sodium thiosulphate using fresh 1% starch solution, a blank titration was also carried out at the same time. The analysis was performed in triplicate. The PV was determined for each sample using the expression below;

$$Peroxide Value\ (\text{mEq}/100\text{g}) = \frac{(\text{T} \times \text{M} \times 1000)}{\text{Weight of Sample (W)}} \qquad (3.4)$$

Where,

T = Titre value Na_2SO_3 (cm^3)

M = Molarity of Na_2SO_3.(mol)

W = Weight of Oil sample (g)

3.3.4 Determination of Acid value

The acid value (AV) determination is done by taken 0.1 - 0.3 g of fat sample or A ml of the extract containing 0.1 -0.3 g of fat in a 100 ml Erlenmeyer flask, Add [10 — A] ml of n-Hexane and 1-2 drops of indicator. Titrate the solution against 0.02N KOH solution. The end point is reached when pink (phenolphthalein) or blue (thymolphthalein) colour persists for 30 seconds and carry out a blank test using A ml of C-M Mixture instead of the extract.

Calculation

Acid value (mg/g) $\times \frac{56.11\ x\ 0.02\ x\ (Vs - Vb)\ x\ F}{w}$ (Badiffu, 1989) (3.5)

Where V_s = titration volume of sample (ml);

V_b = titration volume of blank (ml);

W = weight of fat in the volume of extract used (g);

F = factor of 0.02 KOH solution

Where F = 5

V_f : V_f is the volume of 0.02 N KOH required to neutralize 5 ml of the 0.02 N H_2SO_4 solution.

Molecular weight of KOH= 56.11

Concentration of KOH = 0.02

3.3.5 Determination of Saponification Value of Shea Butter

2g of Shea butter was weighed into a clean dried 250 ml conical flask and 25ml of alcoholic potassium hydroxide (K(OH) $_4$) was added. A reflux condenser was attached and the flask was heated for an hour with frequent shaken. 1 ml of 1% phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5M hydrochloric acid (HCl) until it reached the end point where it turns to colourless. A blank titration was carried out at the same time and under the same condition.

The saponification value was evaluated as

$$Saponification\ Value = \frac{(a-b)\times 0.0205}{W}\times 1000 \quad (3.6)$$

a, b = Constants

W = Weight of Oil sample (g)

The experiment was replicated thrice

3.3.6 Determination of Unsaponifiable Fraction of Shea Butter

The unsaponifiable fraction of Shea butter was determined using AOAC (2000) method. 5g of Shea butter was accurately weighed using metler Toledo balance and mixed in a 250 ml of conical flask. 50 ml of alcoholic potassium hydroxide was added to the mixed Shea butter. The mixture was then boiled under reflux air condenser until saponification was completed. The saponified mixture was transferred while warm to a separating

funnel. The mixture was washed with ethyl alcohol and then with cold water, ensuring that a total of 50 ml of water was used to rinse the flask. 50 ml of petroleum ether was added to mixture and shook vigorously until the layers separate.

The lower soap was transferred into another separating funnel and the ether extraction was repeated thrice using 50 ml portion of petroleum ether. The combined ether extract was first washed thrice with 25 ml of distilled water to ensure the ether extract was free of alkali. The ether solution was transferred to 250 ml beaker and the separator was rinsed with ether. The risings were added to the main solution. The bulk solution was evaporated to about 5 ml and quantitatively transferred using several portion of ether to 50 ml. the ether was then completely evaporated by boiling at 100 ^{o}C for 30 min and 2-3 ml of acetone was added while heating on a water bath until a constant weight was obtained.

The residue was dissolved in 50 ml of warm ethanol which has been neutralised to phenolphthalein end point. The dissolved residue was titrated with 0.02 N NaOH.

`The unsaponifiable fraction is calculated as"

$$\frac{100(A-B)}{W} \qquad (3.7)$$

Where

A = weight in g of the residue

B = weight in g of the FFA in the extract

W = weight in g of the sample

= 0.282 VN

Where

V= volume in ml of standard NaOH solution

N = Normality of standard NaOH solution

3.4 Enzymatic Hydrolysis of Shea Butter Oil

The Shea butter was measured (40g) using 5ml of syringe and poured into a stoppered 250 ml conical flask containing 30 ml of isooctane and 30 ml of phosphate buffer solution with a pH of 7.0 and 0.1g of Lipase to ensure homogenity. This was placed on Magnetic stirrer and with temperature set at 25^{o}C and constant agitation speed of 100 rpm. The solution was allowed for 15 min after which it was stopped. It was poured into separating funnel, 3 distinctive layers observed were separated into 3 separate test tube for further analysis. The hydrolysis was repeated for 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7g of lipase-enzymes, temp of 28, 38, 43, 48^{o}C and the time of 15, 20, 30, 45, 60, 120 and 180 min.

3.5 Experimental Design for the Enzymatic Hydrolysis of SB to Produce FFA

A three-level-four-factor CCD of RSM with design expert (version 13.1.2 Stat-Ease Inc., Minneapolis, MN) was used. The preliminary experiment of Shea butter enzymatic hydrolysis were carried out to determine the lower, mid-point and upper boundary conditions of Lipase-enzyme concentration, Reaction temperature, Reaction time (Rt) and constant Agitation Speed as they affect the FFA content of Shea butter as described in subsections 3.5.1. The boundary conditions obtained were used with the aid of design expert 13.1.2.0 using Central Composite Design as shown in Chapter four, Table 4.3 page 60.

3.5.1 Procedure for the optimization of enzymatic hydrolysis of shea butter for free fatty acid

Central Composite Design (CCD) method of Response Surface Methodology was used for the Design of Experiment (DOE) for the FFA optimization and model was developed for quality processing. The factors used to monitor the quality of the FFA are Liapase concentration [E}, Reaction temperature (T ^{o}C) and Reaction time (t min), at

constant agitation Speed (3 factors) while the response was FFA (1 response) thus giving a total of 20 runs. From the preliminary experiment conducted as described in section 3.2.4.1 the lower, mid-point and upper boundaries shown in chapter four (0.1, 0.4, 0.7 g), (30, 40 and 50 ^{o}C) and (30, 75, 120 min) were chosen. Design expert 13.1.2.0 (2021) package was then used and the experiment designed is as shown in chapter four, Table 4.3.

Free fatty acid was then processed according to the set out conditions in the number of runs and the quality of FFA was measured according to AOAC (1990) methods. To obtain the optimal conditions the FFA was maximized to 0.5634 kg and the FFA was maximized to 56%.

3.5.2 Validation of responses of free fatty acid optimization

The validation of the responses of optimized hydrolysis parameter for FFA was carried out by taking fresh Shea butter and processed according to the optimum conditions obtained from optimization of FFA obtained from design expert 13.2.1. in table 4.5. The software generated the optimum condition of lipase-enzyme concentration [E] 0.529g, RT 34.054 ^{o}C, and Rt 96.534 min**.** The response of FFA (% content) generated by the software was compared with the response obtained experimentally. The deviation and correlation coefficients R^2 were calculated and shown in table 4.6.

3.6 Characterization of Free Fatty Acid for Functional Groups and Chemical Constituents of Hydrolyzed Shea Butter

The procedure for determination of functional groups and chemical constituents using FTIR and GCMS for FFA produced from enzymatic hydrolysis are shown in subsection 3.6.1.

3.6.1 Sample analysis (fourier transform infra-red)

FTIR analyses of FFA produced sfrom was carried out from Central Lab, University of Ilorin, Kwara state Nigeria. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitter). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. Absorbent peaks on the spectrum indicate functional groups (e.g. alkanes, ketones, acid chlorides). Different types of bonds, and thus different functional groups, absorb infrared radiation of different wavelengths.

3.6.2 Sample analysis (gas chromatography and mass spectroscopy)

The FFA produced from the enzymatic hydrolysis was analyzed using a gas chromatography Machine model: 7890A GC system, 5675C Inert MS from Agilent USA hyphenated to a mass spectrophotometer (5675C) with triple axis detector equipped with an auto injector (10ul syringe) was used to determine the constituent composition of Shea butter using Helium gas as a carrier gas at Ilorin, Nigeria. All chromatographic separation of the FFAA was performed on capillary column having specification: length; 30m, internal diameter 0.2um, thickness; 250um, treated with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 250^{o}C, interface temperature; 300^{o}C, pressure; 16.2 psia, out time, 1.8mm, 1ul injector in Split mode with split ratio 1:50 with injection temperature of 300^{o}C the column temperature started at 35^{o}C for 5min and changed to 150^{o}C at the rate of 4^{o}C/min. the temperature was raised to 250^{o}C at the rate of 20^{o}C/min and held for 5min. The total elution was 47.5minutes. Ms Solution software provided by supplier was used to control the system

and to acquire the data; Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NISTII)

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Processed Shea nut to Shea kernel

The 500g of Shea fruits processed on the 4 days of conditioning period with no boiling at all (i.e. Zero min) and dried for 14days were characterize as shown in subsection 4.2 and compared the value with African Organization for Standardization (ARSO, 2017) as follow:

4.2 Characterization of Shea kernel

Table 4.1 shows the physico-chemical property of Shea kernel. The oil yield was 50.20% as compared with the standard value of ≥50 as recommended by GIS, 2015. The free fatty acid and peroxide value were 2.716% and 2.637, respectively. These obtained value for oil yield, FFA and PV fall within standard value recommended (i.e. oil yield ≥50, FFA ≤3 and PV≤5). The kernel was not roasted and after some hours the production of SB was proceeded.

Table 4.1: Physio-chemical property of Shea kernel

Properties	**Obtained value of Shea kernel**	**Standard value of Shea kernel (ARSO)**
Oil Yield	50.20%	≥50
Free Fatty Acid	2.716%	≤3
Peroxide value	2.637	≤5

4.3 Characterization of Shea Butter

The Shea butter produced from the Mechanical Screw Expression method was characterized and shown in table 4.2. This shows the physico-chemical properties of the SB as follows. The free fatty acid (FFA) was 12.44%**,** this showed that FFA is slightly high and high FFA is not suitable for cosmetic and food uses because it produces irritation on the tongue and in the throat, (Ajala *et al*., 2015). Therefore, it may be more

suitable for different purposes. Similarly, the acid values of the samples were 15.9616 mgKOH/g of oil. The higher acid value showed the extent of decomposition of the glycerides in the SB by lipase or heat and light. It is also an indication for the condition and edibility of the oil. The iodine value is 83.39g I2/100 g oil, these results showed that SB is more saturated, with higher degree of unsaponification and shorter shelf-life (Ajala *et al*., 2015), because the FFA and iodine value is the measure of the degree of unsaturation of vegetable oil, (Ajala *et al*., 2015). The changes in these properties can therefore be used in monitoring deterioration of SB, (Ajala *et al*., 2015). The peroxide values obtained was 7.456 (mg/100g) for SB. The recommended value for cosmetic and food uses is less than 10 mg/100g, (Ajala *et al*., 2015). The saponification values obtained in this study are 189.93 (mgKOH/g oil). This indicates that unsaturated fatty acid is present (Ajala *et al*., 2015).

Table 4.2: Characterization of Shea butter from mechanical screw expression

Properties	**Shea butter from Mechanical Screw Expression**
Acid value	15.9616
Specific gravity	0.915
Free Fatty Acid	12.44%
Peroxide value	7.456
Saponification Value	189.93
Iodine value(g/100g)	83.39

4.4 Preliminary Production of FFA from Shea Butter via Enzymatic Hydrolysis

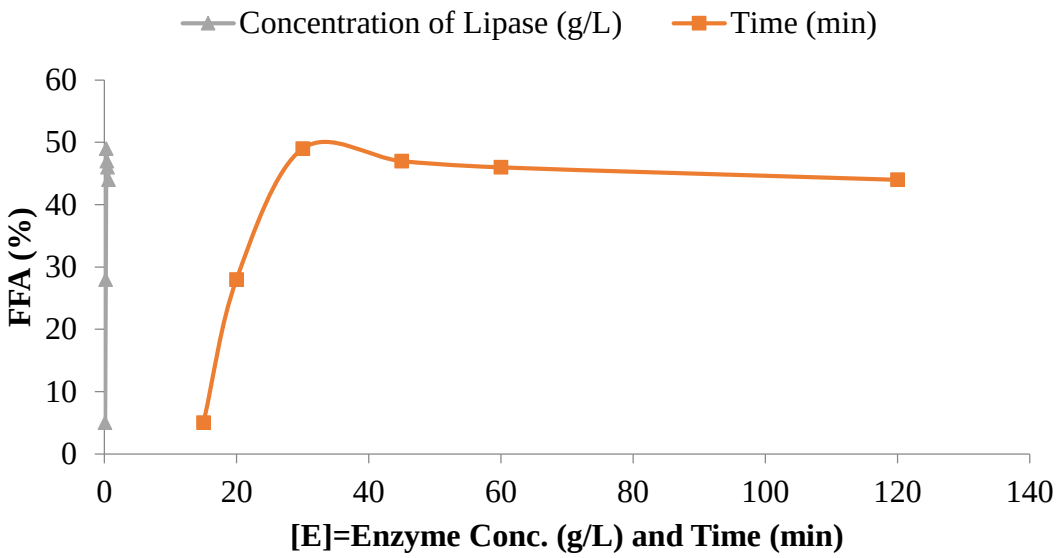

Figure 4.1: Effect of Enzyme-Lipase concentration on the hydrolysis Time of 50g Shea butter to produce FFA at constant temperature of 30 ^{o}C and agitation speed of 100rpm

Figure 4.1 shows that as Enzyme-Lipase concentration increases over time, the rate of formation of FFA (%) also increases. This clearly indicate that high lipase-enzyme concentration lower the activation energy required for the reactive molecular collision to occur between the enzyme and the Shea butter by providing surface contact on which the reaction will proceed fast. At the Enzyme-Lipase concentration of 0.3 g/L the FFA produce is the highest and shortly after that any increase in the enzyme-lipase concentration, lowers the percentage yield of FFA. This is probably due to equilibrium between the enzyme-lipase concentration and the molecules of Shea butter or may result as a function of saturated free fatty acid formation (Ajala *et al.*, 2015).

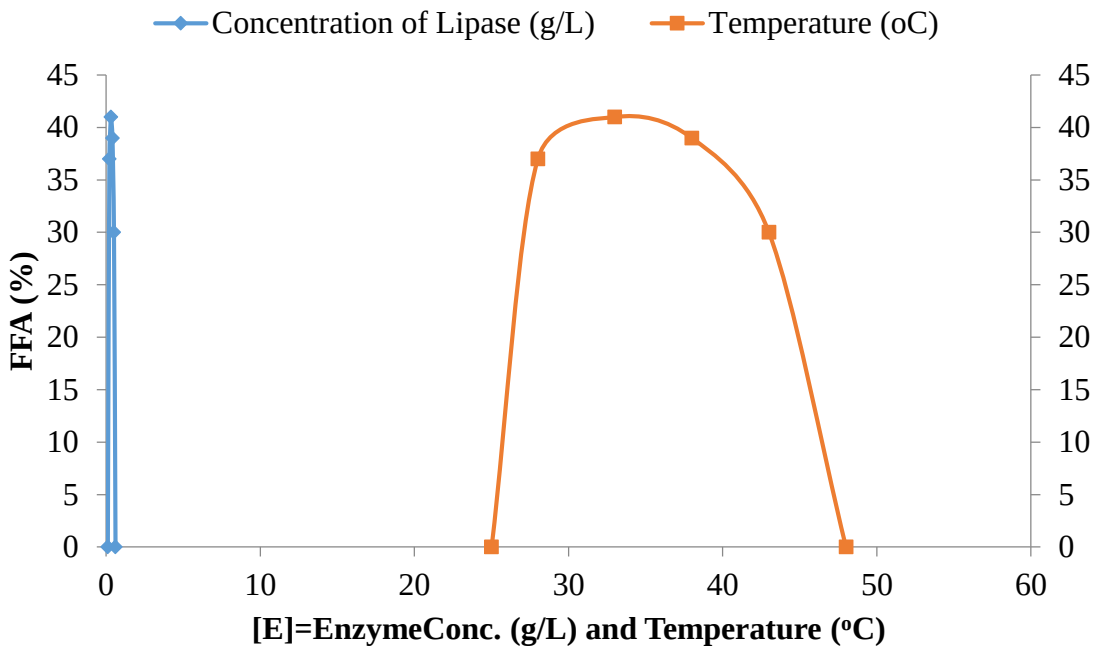

Figure 4.2: Effect of hydrolysis Temperature on lipase-enzyme concentration of 50g Shea butter to produce FFA at constant time of 60 min and agitation speed of 100rpm

The solubility of a solute in solvent at a given temperature is the number of moles of the solute necessary to saturate 1dm^3 (or 1kg) of the solvent at that temperature. Figure 4.2 shows that the solubility of the lipase-enzyme concentration increases steadily with temperature rise as rate formation of FFA increases. However, at a favorably temperature of 33 ^{o}C the rate formation of FFA (41%) is highest; where the activities of lipase-enzyme is double, while at the temperature slightly above 33 ^{o}C the lipase-enzyme activities were reduced and at the temperature of 48 ^{o}C they become inactive, may be dead (Ajala *et al.*, 2015).

4.5 Optimization of Process Parameters for the Product Formation of FFA

The boundary conditions obtained in figure 4.1 and 4.2 were used in DOE for enzymatic hydrolysis of Shea butter to produce FFA whose responses were determined as shown in Table 4.3.

4.6 Responses of Shea Butter from Central Composite Design

The responses of FFA obtained as function of amount of lipase-enzyme concentration, reaction temperature and reaction time from the DOE of FFA processing using central composite design method is shown in Table 4.3.

Table 4.3: Experimental design of enzymatic hydrolysis of Shea butter and yield of FFA (%) at constant Agitation Speed of 100rpm using CCD

Std	Run	Factor A: Wt% of Lipase	Factor B: Temperature (°C)	Factor C: Time (min)	%Yield of FFA	
					Actual Value	Predicted Value
10	1	0.7	35	60	44.20	43.99
18	2	0.3	35	90	48.00	48.94
9	3	0.1	35	90	32.73	32.36
5	4	0.2	30	105	45.50	45.63
6	5	0.6	35	120	56.20	55.62
17	6	0.3	35	90	48.50	48.94
15	7	0.4	40	75	45.80	45.88
3	8	0.2	45	45	22.90	23.31
8	9	0.6	45	120	33.82	34.74
20	10	0.4	40	75	45.80	45.88
1	11	0.3	30	30	30.50	29.88
12	12	0.4	50	105	23.50	23.18
19	13	0.4	40	75	45.80	45.88
14	14	0.4	40	75	45.80	45.88
13	15	0.4	40	75	45.80	45.88
11	16	0.5	35	60	50.00	48.68
2	17	0.6	30	30	34.00	34.96
4	18	0.6	45	45	31.50	31.26
16	19	0.4	40	75	45.80	45.88
7	20	0.3	45	120	31.50	30.89

The %FFA obtained from the enzymatic hydrolysis is the response (Y) with the minimum obtainable as 23.50% from Exp. Run 20 as shown in Table 4.4.

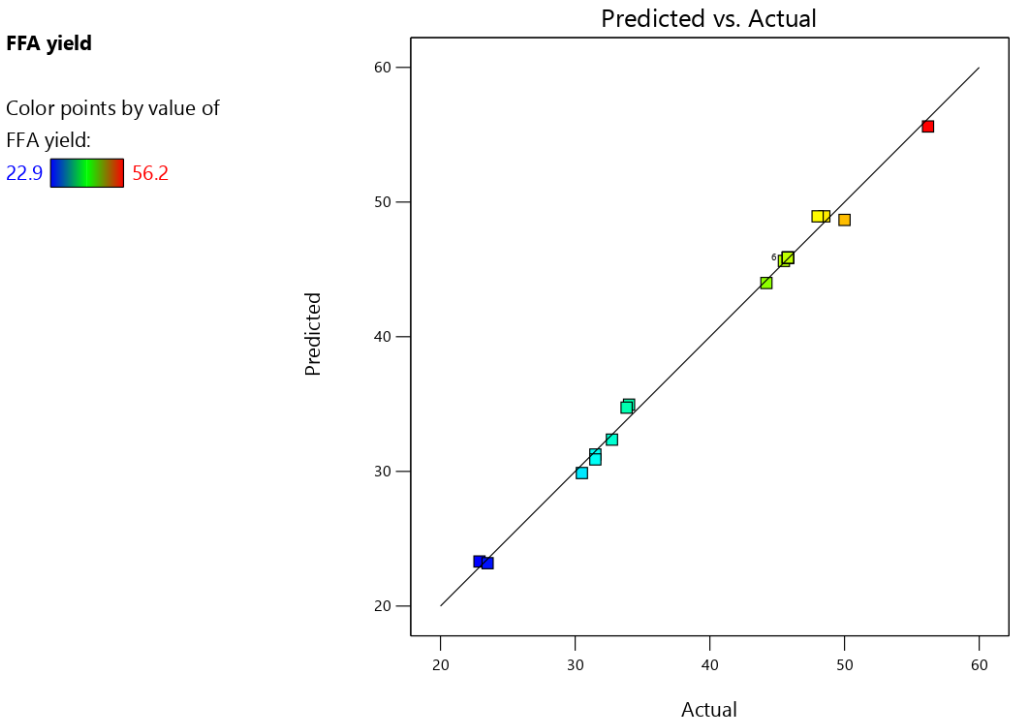

Figure 4.3: Plot of Actual versus Predicted value of % yield of FFA

4.7 Final Model Equation in Terms of Actual Factors

The regression Eq. (4.1) for the determination of predicted values of output parameter (FFA) is given as:

FFA yield=-117.21013 + 153.96675*wt of Lipase + 5.40171*Temp + 1.08506*Time - 0.749055*wt of Lipase*Temp + 0.079316*wt of Lipase*Time - 0.013163*Temp*Time - 129.95662*wt of Lipase^2 - 0.068263*Temp^2 - 0.002994*Time^2 (4.1)

Where A, B & C are the variables in term of wt % of Lipase=enzyme, temperature (^{o}C) and time (min) respectively.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

4.8 Optimization Statistic

The p-value (0.0001) of the model shows that the Model F-value of 296.64 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. The Linear model: the wt. % of Lipase-enzyme (A), temperature (B) and time (C) each of the variables gave p value of <0.05 as shown in Table 4.4; an indication that in their linear form, they are highly significant. While the values greater than 0.1000 indicate the model terms are not significant. Non-linear model: The interactions effect between wt. % of Lipase-enzyme (A) and temperature (B) and temperature (B) and Time (C) were also significant with $p < 0.05$. The quadratic effect of wt. % of Lipase-enzyme (A), temperature (B) and time (C) were all significant with $p < 0.0001$ (Table 4.4). The Lack of Fit F-value of 73.37 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise. Significant lack of fit is bad -- we want the model to fit.

Table 4.4: Sum of squares is Type III–Partial ANOVA for Quadratic model of Response 1: FFA yield

Factor	**% Yield of FFA**		
	Coefficient	**F-value**	**P-value**
Model		296.64	< 0.0001
A	153.96675	340.03	< 0.0001
B	5.40171	1334.27	< 0.0001
C	1.08506	316.20	< 0.0001
AB	-0.749055	10.60	0.0086
BC	- 0.013163	2.99	0.1145
AC	+ 0.079316	112.66	< 0.0001
A^2	- 129.95662	305.06	< 0.0001
B^2	- 0.068263	75.21	< 0.0001
C^2	- 0.002994	70.97	< 0.0001
Lack of fit		73.37	<0.0001

From Table 4.5, the value of regression coefficient (R^2) is 0.9963 which is significant for the model, the Predicted R^2 of 0.9383 is in reasonable agreement with the Adjusted R^2 of 0.9929; i.e. the difference is less than 0.2, which also corroborates the reliability of the model. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 58.071 indicates an adequate signal. This model can be used to navigate the design space.

Table 4.5: Coefficient of Regression Analysis for hydrolysis of Shea Butter

Regression	**Value**
R-Square	0.9963
Adjusted R-Square	0.9929
Predicted R-Square	0.9383
Adeq Precision	58.071
Coefficient of Variance	1.96%
Std. Dev.	0.7899
Mean	40.38

4.9 Optimization Analysis

The constraints which provided in the lower and upper limit were as (A): wt % of lipase-enzyme of 0.2 to 0.6, (B): Temperature of 34.054 to 45.946 and (C): Time of 31.0211 to 120.26. The optimum value generated by the DOE are 0.40wt of Lipase-enzyme, Temperature of 40 ^oC, Time of 75 min and FFA yield of 56.20%.

Table 4.6: Optimal Parameters for enzymatic hydrolysis of High FFA for SB

Parameters	**Optimum Level**	**Low Level**	**High Level**
A: wt. % Lipase-enzyme	0.40	0.20	0.60
B: Temperature (^oC)	40.00	34.054	45.946
C: Time (min)	75.00	31.0211	120.26

4.10 Effect of wt. % of lipase-enzyme (A) and temperature (B) on SB to yield %FFA

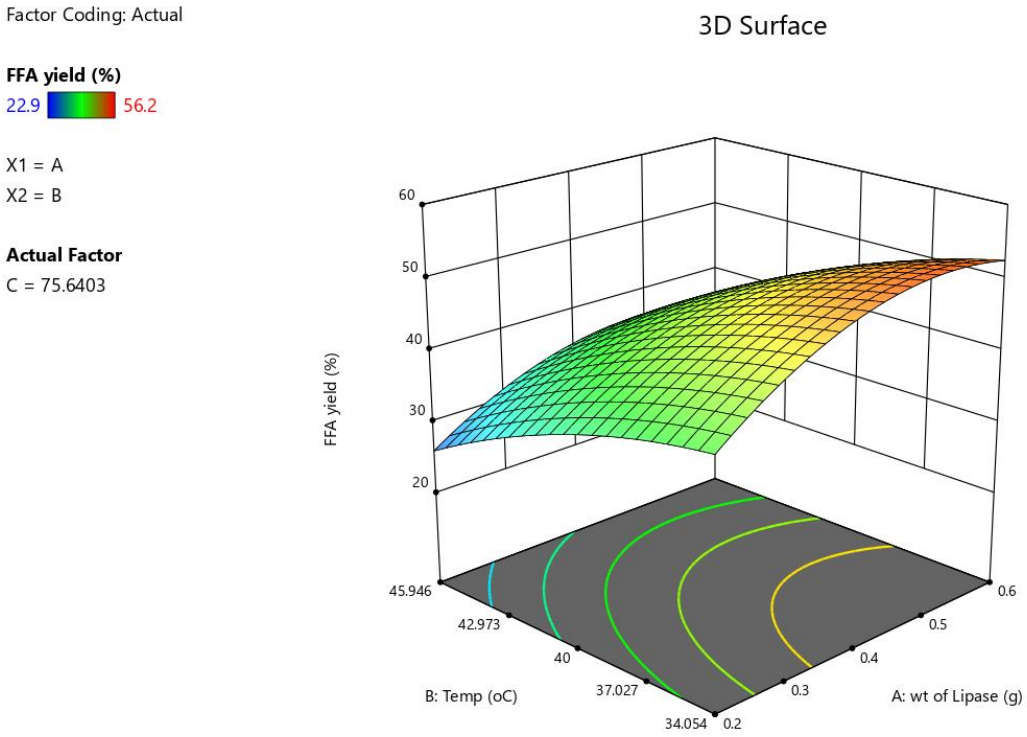

Figure 4.4: Effect of Wt% of Lipase (g) and Temp (^{o}C) on the hydrolysis of % yield of FFA

Figures 4.4 show the effect of wt% of Lipase-enzyme as it interacts with temperature to yield % FFA. The figures showed that low % yield of FFA of 25.98% was attained at high temperature of 45.95 ^{o}C and low wt. % Lipase-enzyme of 0.2g. While maintaining the high temperature of 45.95 ^{o}C and increasing the wt % of lipase=enzyme increases the % yield of FFA to 34.62%. This indicates that maintaining the high temperature of 45.95 and increasing the wt% of lipase=enzyme increases the FFA%. This means that wt% lipase=enzyme is adequately enough at that high temperature, so the solubility is also high. On the other hand, lowering the temperature below the optimum (34.054 ^{o}C) and also lowering the wt% of lipase (0.2g) increases the % yield of FFA to 40.31%. While, the highest % yield of FFA of 52.51% was achieved at a moderate temperature of 34.054 ^{o}C and high wt. % Lipase-enzyme of 0.6g. This is because moderate temperature increases the solubility of Lipase-enzyme in Shea butter. This indicates that

moderate temperature of 34.054 ^{o}C below the optimum (40 ^{o}C) at high wt % of lipase=enzyme favours high FFA%.

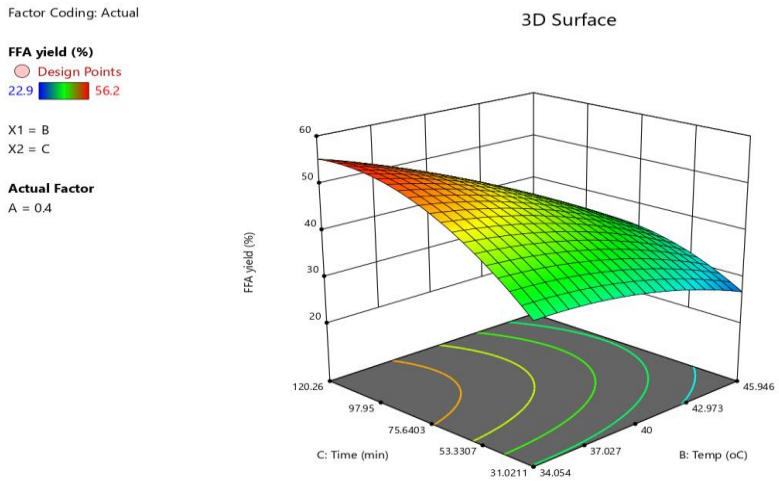

Figure 4.5: Effect of Temperature (^{o}C) and Time (min) on the hydrolysis of % yield of FFA

Figures 4.5 show the effect of temperature as it interacts with time of enzymatic hydrolysis to yield %FFA. The figure showed that at moderate temperature of 34.054 ^{o}C below the optimum 40 ^{o}C and short hydrolysis time of 31.02 min, the % yield of FFA was 36.03%. While, above the optimum temperature of 45.95 ^{o}C and constant short hydrolysis time of 31.02 min reduces the % yield of FFA to 26.90%. This means that at this high temperature of 45.95 ^{o}C above the optimum makes the lipase=enzyme becomes inactive or possibly dead. However, maintaining the moderate temperature of 34.054 ^{o}C at longer hydrolysis time of 120.26 min, the %FFA yield more to 55.98%. This implies that high wt% of Lipase-enzyme at moderate temperature can survive for longer hydrolysis time thereby resulting to high % yield of FFA in the Shea butter. Therefore, this indicates that temperature below and above the optimum effect the performance of lipase=enzyme**.**

4.11 Effect of wt. % of lipase-enzyme (A) and time (C) on the hydrolysis of SB to yield %FFA

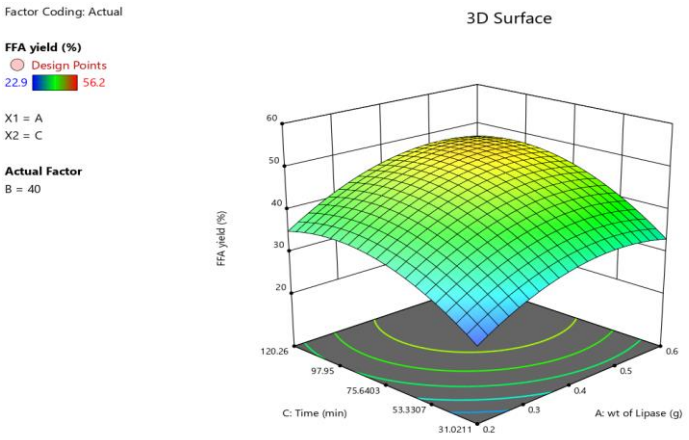

Figure 4.6: Effect of Wt% of Lipase (g/L) and Time (min) on the hydrolysis of SB to yield %FFA

Figure 4.6 show the effect of wt% of Lipase=enzyme as it interacts with time to yield % FFA. The figures showed that at low wt % of lipase=enzyme of 0.2g and low hydrolysis of time of 31.02 min, the % yield of FFA stood at 24.18%. While, at high wt% of lipase-enzyme (0.6g) and low constant hydrolysis time of 31.02 min the % yield increases to 33.18%. This implies that low amount of wt % of lipase=enzyme of 0.2g does not completely hydrolyze the Shea butter and at high of 0.6g of the lipase=enzyme it is good enough to completely hydrolyze the Shea butter. However, at high hydrolysis time of 120.26 min and low wt % of lipase=enzyme the % yield of FFA increases to 35.20%. While, maintaining the high hydrolysis time of 120.26 min and increasing the wt. % Lipase-enzyme to 0.6g % yield of FFA equally increases to 46.85% for the hydrolysis of Shea Butter. This means that high wt % of lipase=enzyme will require a longer hydrolysis time to hydrolyze the Shea butter.

4.12 Optimization Validation

Table 4.7: Optimization validation of enzymatic hydrolysis of High %FFA from SB

Validation	**A**	**B**	**C**	**FFA%**
Predicted Result from DOE	0.487	34.966	107.404	56.334
Experimental Result				56.00
Percentage error				0.596

The constraints which provided in the lower and upper limit were as A: wt of enzyme-lipase 0.2 to 0.6, B: Temp. 34.054 to 45.946 and Time of 31.0211 to 120.26. 100 Solutions were found with the best desirability of 1.000, amount of 0.487 wt of enzyme-Lipase, Temperature of 34.966 ^{o}C, Time of 107.404 min and FFA yield of 56.334%. The model predictions were validated by repeating the experiment in triplicate using the predicted optimal values. The %yield of FFA obtained was an average value of 56.00%, which is very close to the model prediction. Table 4.7 also shows the %error of 0.596%, which is close to the allowable limit of 0.5%. This result showed that the predicted result is in agreement with the experimental value. This showed that the model is reproducible and sufficiently described the hydrolysis process.

4.13 Reaction Kinetics of the Enzymatic Hydrolysis of Shea butter to Produce FFA

There are several factors that affect the performance of lipase-enzyme in the hydrolysis process namely;

(1). Enzyme concentration

(2). Temperature

(3). Substrate concentration

(4). Product concentration

(5). pH

(6). Activation

(7). Inhibition and population

(8). Induction and Repression

(9). Catalyst Modification

(10). Compartmentalization

(1). Enzyme concentration: The velocity or reaction rate is directly proportional to the enzyme concentration [E], when sufficient substrate concentration [S] is present.

Or when velocity of reaction is increased proportionately with the concentration of enzyme, provided substrate concentration is unlimited.

Mathematically;

$$V = \frac{d[P]}{dt}\alpha[E]$$

$$V = \frac{d[P]}{dt} = [E][S] \quad (4.1)$$

(2). Temperature: as the velocity reaction increases when the temperature of the medium is increase, reaches a maximum and then falls. The temperature at which maximum concentration of the substrate is converted the product per unit time is called optimum temperature.

$$V = \frac{d[P]}{dt}\alpha\, T$$

$$V = \frac{d[P]}{dt} = T[S] \quad (4.2)$$

If we put equation (1) into (2) we have;

$$[E][S] = T[S]$$

$$[E] = T \quad (4.3)$$

Equation 4.3 means the optimum temperature which enzyme concentration [E] can resist, the product formation per unit time (d[P]/dt) at this equilibrium point is maximum and this is graphically illustrated as shown in figure 4.7.

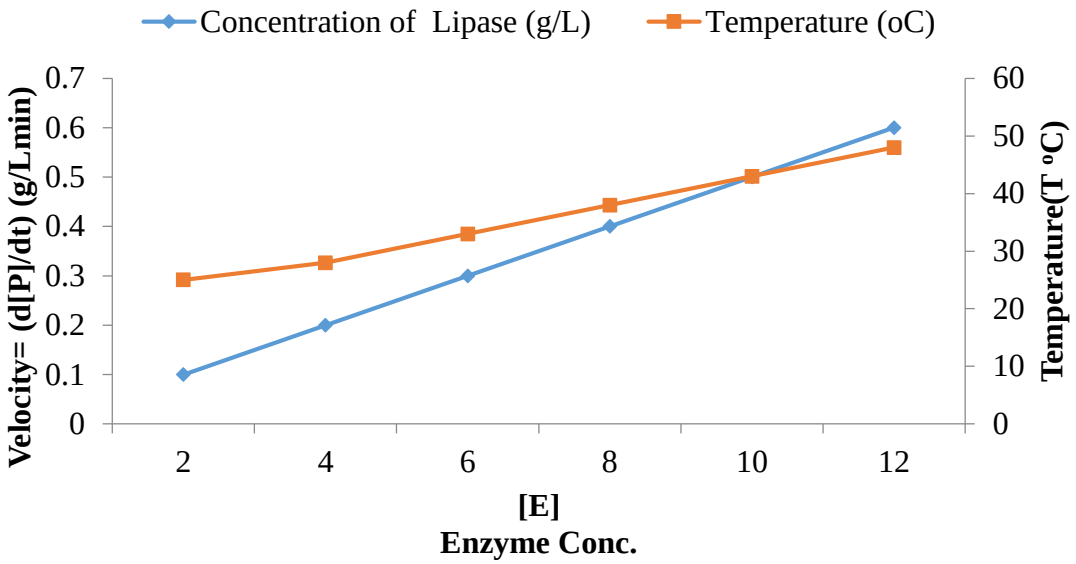

Figure 4.7: Combine effect of Lipase-enzyme concentration and Temperature on product formation

(3). Product concentration: Also here, the velocity or reaction rate is directly proportional to the product concentration [S], provided that sufficient Enzyme concentration [E] is present.

Michaelis-Menten relate this to velocity equal to dissociation rate of reactant and equal to product formation rate.

i.e. $velocity = rate\ of\ dissociation = rate\ of\ formation$ (4.4)

He further explain this by establish mathematical relation between V_o, V_{max} and K_m

$$[E] + [S] \underset{k_r}{\overset{k_f}{\rightleftharpoons}} [ES] \xrightarrow{k_{cat}} [E] + P \quad (4.5)$$

At equilibrium [ES] is constant during the enzymation reaction

[ES] = Dissociation of reactant = Formation of product

Therefore, equation 4.4 can be writen as $Rate = v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$

From 4.5 $k_f[E][S] = k_r[ES] + k_{cat}[ES]$ (4.6)

$$\frac{[E][S]}{[ES]} = \frac{k_r - k_{cat}}{k_f} = k_m = Michaelis - Menten\ constant \quad (4.7)$$

Assumptions

(1) If equation 4.7 is true when free enzyme molecules exist (i.e. not bonded to the subtrate) $Total[E] = [E]_i + [ES]$, $[E]_i = 1$ First order reaction where there is linear increase in velocity with increase in subtrate concentration

Then, $Total\ [E] = 1 + [ES]$ (4.8)

(2) If all enzyme were bonded to the subtrate

$[E]_i = 0$, Second order reaction where there no change in velocity with subtrate concentration

Then, $Total = [E] = [ES]$ (4.9)

If the first assumption is valid, put equation 4.8 into 4.7

$$\frac{([E]_i + [ES])[S]}{[ES]} = k_m$$

$$\frac{([E]_i[S]}{[ES]} = k_m + [S] \quad (4.10)$$

Also, if the velocity depend on breakdown of [ES] complex, then

$$\mathrm{V} = \mathrm{V_{max}}[\mathrm{ES}]$$

Then, $\frac{\mathrm{V}}{\mathrm{V_{max}}} = [\mathrm{ES}]$ (4.11)

And $[\mathrm{E_i}] = 1$

$$V = \frac{\mathrm{V_{max}}}{k_m + [S]}[S] \quad (4.12)$$

If the second assumption is valid, put equation 4.9 into 4.7, note that $[\mathrm{E_i}] = 0$, [E]=[ES]

$$\frac{[E][S]}{[ES]} = k_m$$

$$[S] = k_m \quad (4.13)$$

Also $\mathrm{V} = \mathrm{k_{cat}}[\mathrm{ES}]$

$$\frac{\mathrm{V}}{[\mathrm{E}]} = \mathrm{k_{cat}} \quad (4.14)$$

$$\frac{\mathrm{V}}{[\mathrm{E}]} = \mathrm{k_{cat}}$$

At $V_{max} = V$ (4.15)

Put equation 4.15 into 4.12

$V_{max} = \frac{V_{max}}{k_m + [S]}[S]$

$k_m = 0$ (4.16)

(3) Also, when $V = \frac{V_{max}}{2}$ (4.17)

Put equation 4.17 into 4.12

$k_m = [S]$ (4.18)

Graphically the vital equation can be shown,

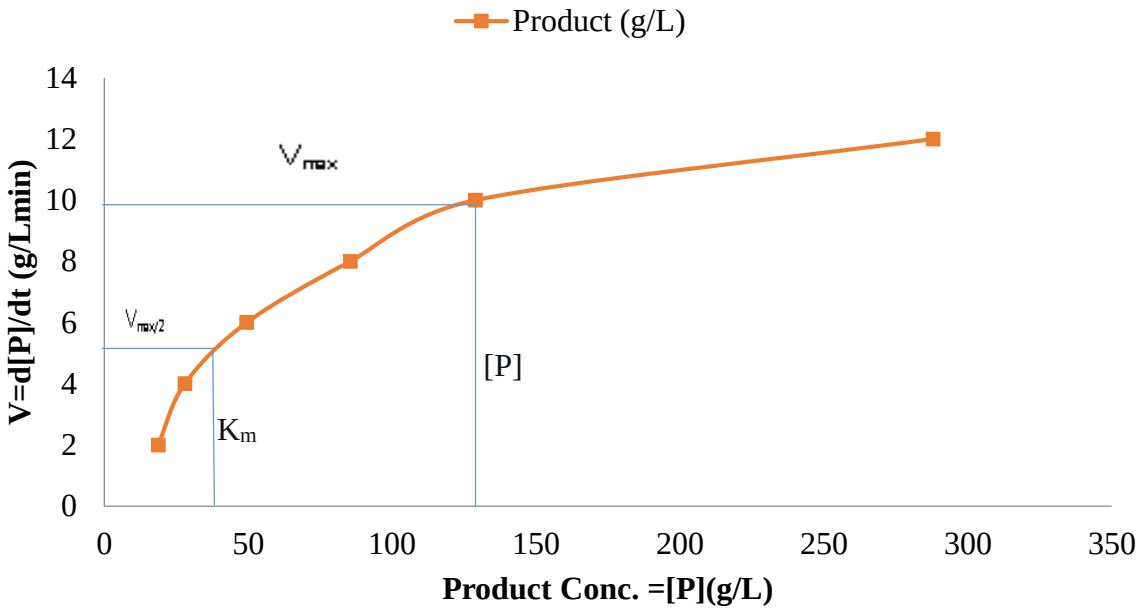

Figure 4.8: Plot of Velocity (g/Lmin) against product concentration (g/L)

Calculation:

When V_{max}=10 (g/Lmin) then [P] = 129 (g/L),

When $V_{max}/2$ = 5 then k_m = 3875

$k_{cat} = 0.25$,

If k_f=k_r, then $\frac{k_r}{k_f} = 1$, $k_f = \frac{k_m + 1}{k_{cat}} = \frac{38.75+1}{0.25} = 8$ also k_r =8

4.14 Characterization of Free Fatty Acid extracted by enzymatic hydrolysis

4.14.1 FTIR

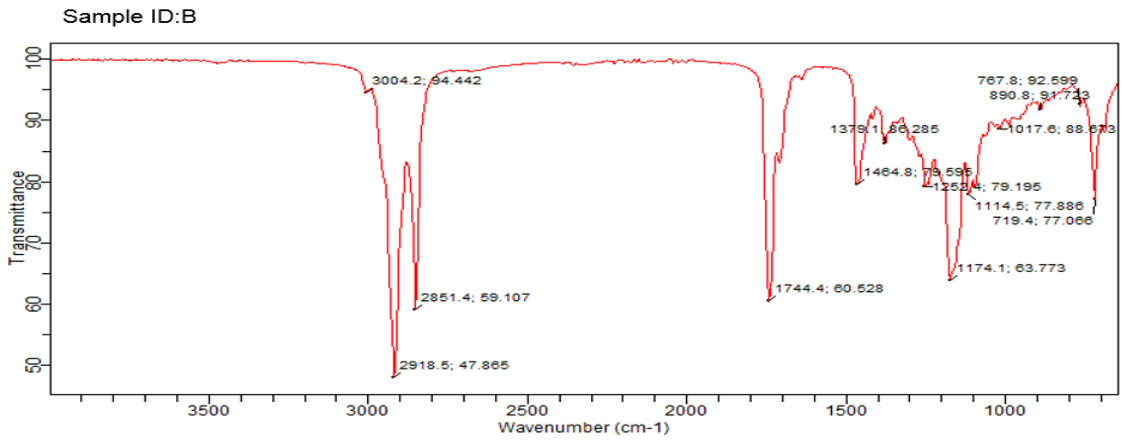

Figure 4.9: FTIR spectra of FFA from Enzymatic Hydrolysis of Shea Butter

Figure 4.9 shows the FTIR spectra of the free fatty acid samples. The spectrum for each sample shows identical pattern with major peaks at frequency of 3004-1379 cm^{-1} while the finger prints are within 1252-900 cm^{-1}. The peak observed at 3004 cm^{-1} is ascribed to the presence of unsaturated hydrocarbon chain while 2914.8 and 2851.4 are attributed to C-H bond, signifying methylene group. The frequency of 1744.4 cm^{-1} is attributed C=O bond, suggesting the ester carbonyl functional group of the triglycerides. According to Elijah *et al.* (2019), this frequency is a typical characteristic of vegetable oils with saturated fatty acids content. The peaks around 1640-1462$cm^{-1}$are ascribed to C=C indicating the presence of alkenes/aromatic hydrocarbons. Similarly, the frequency observed at 1379 $cm^{-1}$is also due to C=C bond of unsaturated hydrocarbon chain. In the finger print region, vibration at 1252, 1174 and 1114 are assigned to C-O stretching of the ester group while the 719$cm^{-1}$is due to aromatic C-H bending vibration (Ajala *et al.,* 2016)

4.12.2 GC-MS

Table 4.8 shows the free fatty acids produced from optimized enzymatic hydrolysis with 55.97% of Stearic acid, 40.03% of Oleic acid and 4.00% of alpha, -Amyrin as the bio-active. The fatty acids obtained from the GC-MS fragments are described as follows; Oleic Acid (also known as cis-9-Octadecenoic acid, cis-Oleic acid, oleate, Elaidoic acid, Metaupon, Delsauere), an unsaturated fatty acid most widely distributed and abundant in nature. It is used commercially in the preparation of oleates and lotions, and as a pharmaceutical solvent. Oleic Acid's high lipid count makes it a great moisturizer, and a number of cosmetic companies add it to lotions and soaps in order to boost their ability to nourish the skin.

Stearic acid was also obtained (stearic acid is among the most common saturated fatty acids), is a saturated fatty acid with an 18-carbon chain and has the IUPAC name octadecanoic acid, Stearic acid is mainly used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Soaps are not made directly from stearic acid, but indirectly by saponification of triglycerides consisting of stearic acid esters. Esters of stearic acid with ethylene glycol, glycol stearate, and glycol distearate are used to produce a pearly effect in shampoos, soaps, and other cosmetic products. They are added to the product in molten form and allowed to crystallize under controlled conditions. Detergents are obtained from amides and quaternary alkylammonium derivatives of stearic acid Surfactants, cosmetics and personal hygiene products are infact prospects of stearic acid, Warra A.A, (2015). Another fatty acid obtained is α-Amyrin which are bioactive and common name for 10-undecenoic ly in the pharmaceutical industries to treat anti-inflammation, anti-bacterial and anti-fungi, Warra A.A, (2015).

Table 4.8: Free fatty acids component and the amount in %

Free Fatty Acid	**Molecular formula**	**Amount**
Stearic acid	$C_{18}H_{32}O_2$	51.21
Oleic acid	$C_{18}H_{34}O_2$	45.43
α-Amyrin	$C_{30}H_{50}O$	3.36%

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. Extraction of shea butter from shea kernel was successfully done using mechanical screw expression method.
2. Enzymatic production of free fatty acid (FFA) from shea butter was successfully carried out and a yield of 56.33% was achieved at the optimum condition of 0.487 g/L of Lipase-enzyme concentration, 34.96^{o}C of temperature and 107 min using 0.05 g/L of shea butter at agitation speed of 100 rpm.
3. The kinetics variables generated from the enzymatic hydrolysis reaction showed a significant effect on the % increase in FFA. The reaction rate constant, k_r obtained is 8 and the model can successfully be employed in the hydrolysis of Shea butter process for industry to increase the FFA content.
4. The physiochemical properties carried out indicated that the shea kernel and shea butter extracted closely aligns with the standard of saponification value, acid value, iodine value and specific gravity. The GC-MS showed free fatty acids produced from optimized enzymatic hydrolysis with 55.97% of Stearic acid, 40.03% of Oleic acid and 4.00% of alpha, -Amyrin as the bio-active.

5.2 Recommendations

The various factors that affects the kinetic of Lipase such as pH, activation, Inhibition and population, Induction and Repression, Catalyst Modification and Compartmentalization should be considered for the future research purpose.

5.3 Contribution to Knowledge

Mechanical screw extraction method of Shea butter production predominantly produces Shea butter with high value of free fatty acid in Nigeria. Enzymatic hydrolysis of Shea Butter equally leads to production of free fatty acid which is highly demanded by the Pharmaceutical and Cosmetics industries. This research contributes the following improvement into the quality of traditionally produced Shea butter.

1. This work reports the effect of Shea nuts/Shea kernel optimization on the yield of 56.33% and quality of Shea butter as against the conventional Shea nuts/kernel processing methods.
2. The study shows the effects of optimization of some selected variables that influences mechanical extraction process on the yield and quality of Shea butter.
3. The work report similarities physicochemical properties between free fatty acid produced before and after optimization of Shea kernel.
4. The study report yield, quality and kinetic optimum properties of free fatty acid from enzymatic hydrolysis.

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APPENDIX

The effect of Time on the concentration of enzyme-Lipase in the hydrolysis of 50g Shea butter to produce FFA at constant temperature of 30 ^{o}C and agitation of 100rpm

S/No.	Concentration of Lipase (g/L)	Time (min)	FFA (%l)
1	0.1	15	5
2	0.2	20	28
3	0.3	30	49
4	0.4	45	47
5	0.5	60	46
6	0.6	120	44
7	0.0	1,440	1.81

The effect of Temperature on concentration of enzyme-Lipase in the hydrolysis of 50g Shea butter to produce FFA at constant time of 60 min and agitation of 100rpm

S/No.	Concentration of Lipase (g/L)	Temperature (^{o}C)	FFA (%)
1	0.1	25	0
2	0.2	28	37
3	0.3	33	41
4	0.4	38	39
5	0.5	43	30
6	0.6	48	0

Combine effect of concentration of enzyme-Lipase, Temperature and Time of hydrolysis of 0.050 (g/L) of Shea butter to produce Concentration of FFA at constant agitation speed of 100rpm

S/No.	Concentration of Lipase (g/L)	Temperature (^{o}C)	Time (min)	V (d[P]/dt)
1.	0.1	25	15	2
2.	0.2	28	20	4
3.	0.3	33	30	6
4.	0.4	38	45	8
5.	0.5	43	60	10
6.	0.6	48	120	12